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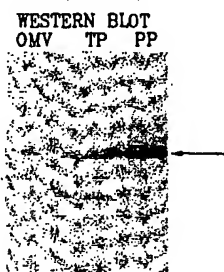
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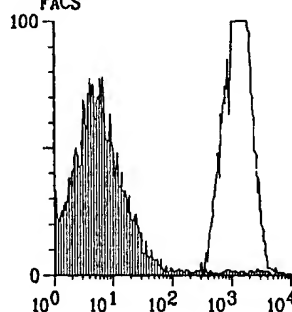
A
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PURIFICATION
MI 919



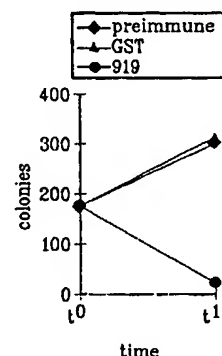
B
919 (48 kDa)
WESTERN BLOT
OMV TP PP



C
919 (48 kDa)
FACS



D
919 (48 kDa)
BACTERICIDAL ASSAY



919 (48 kDa)
ELISA assay: positive

E

(57) Abstract

The invention provides methods of obtaining immunogenic proteins from genomic sequences including *Neisseria*, including the amino acid sequences and the corresponding nucleotide sequences, as well as the genomic sequence of *Neisseria meningitidis B*. The proteins so obtained are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.

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NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

This application claims priority to provisional U.S. application serial no. 60/132,068, filed 30 April 1999; PCT/US99/23573, filed 8 October 1999 (to be published April 2000); and Great Britain application serial no. GB-0004695.3, filed 28 February 2000.

This invention relates to methods of obtaining antigens and immunogens, the antigens and immunogens so obtained, and nucleic acids from the bacterial species: *Neisseria meningitidis*. In particular, it relates to genomic sequences from the bacterium; more particularly its "B" serogroup.

BACKGROUND

Neisseria meningitidis is a non-motile, gram negative diplococcus human pathogen. It colonizes the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N. gonorrhoea*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

N. meningitidis causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks. (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N. meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the

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United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants (e.g., Morbidity and Mortality weekly report, Vol. 46, No. RR-5 (1997)). This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H. influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease". In: *New Generation Vaccines, supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A (menA) and C (menC) (*Vaccine* 10:691-698)).

Meningococcus B (MenB) remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the MenB capsular polysaccharide is a polymer of $\alpha(2-8)$ -linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to MenB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (e.g.,

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Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (e.g., Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (e.g., EP-A-0467714, WO96/29412), but this is by no means complete. The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable or at least are more antigenically conserved than other and more variable regions. Thus, those antigenic sequences that are more highly conserved are preferred sequences. Those sequences specific to *Neisseria meningitidis* or *Neisseria gonorrhoeae* that are more highly conserved are further preferred sequences. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*. The identification of sequences from the bacterium will also facilitate the production of biological probes, particularly organism-specific probes.

It is thus an object of the invention is to provide Neisserial DNA sequences which (1) encode proteins predicted and/or shown to be antigenic or immunogenic, (2) can be used as probes or amplification primers, and (3) can be analyzed by bioinformatics.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.

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Fig. 4 illustrates the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 6 illustrates the products of protein expression and purification of the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 7 illustrates the products of protein expression and purification of the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 8 illustrates the products of protein expression and purification of the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 9 illustrates the products of protein expression and purification of the predicted ORF 406 as cloned and expressed in *E. coli*.

Fig. 10 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 11 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 12 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 13 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 14 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 15 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 16 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 17 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 18 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 406 as cloned and expressed in *E. coli*.

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THE INVENTION

The first complete sequence of the genome of *N. meningitidis* was disclosed as 961 partial contiguous nucleotide sequences, shown as SEQ ID NOs:1-961 of co-owned PCT/US99/23573 (the '573 application), filed 8 October 1999 (to be published April 2000). A single sequence full length genome of *N. meningitidis* was also disclosed as SEQ ID NO. 1068 of the '573 application. The invention is based on a full length genome of *N. meningitidis* which appears as SEQ ID NO. 1 in the present application as Appendix A hereto. The 961 sequences of the '573 application represent substantially the whole genome of serotype B of *N. meningitidis* (>99.98%). There is partial overlap between some of the 961 contiguous sequences ("contigs") shown in the 961 sequences, which overlap was used to construct the single full length sequence shown in SEQ ID NO. 1 in Appendix A hereto, using the TIGR Assembler [G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995)]. Some of the nucleotides in the contigs had been previously released. (See ftp://11ftp.tigr.org/pub/data/n_meningitidis on the world-wide web or "WWW"). The coordinates of the 2508 released sequences in the present contigs are presented in Appendix A of the '573 application. These data include the contig number (or i.d.) as presented in the first column; the name of the sequence as found on WWW is in the second column; with the coordinates of the contigs in the third and fourth columns, respectively. The sequences of certain MenB ORFs presented in Appendix B of the '573 application feature in International Patent Application filed by Chiron SpA on October 9, 1998 (PCT/IB98/01665) and January 14, 1999 (PCT/IB99/00103) respectively. Appendix B hereto provides a listing of 2158 open reading frames contained within the full length sequence found in SEQ ID NO. 1 in Appendix A hereto. The information set forth in Appendix B hereto includes the "NMB" name of the sequence, the putative translation product, and the beginning and ending nucleotide positions within SEQ ID NO. 1 which comprise the open reading frames. These open reading frames are referred to herein as the "NMB open reading frames".

In a first aspect, the invention provides nucleic acid including the *N. meningitidis* nucleotide sequence shown in SEQ ID NO. 1 in Appendix A hereto. It also provides nucleic acid comprising sequences having sequence identity to the nucleotide sequence disclosed herein. Depending on the particular sequence, the degree of sequence identity is preferably

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greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). These sequences include, for instance, mutants and allelic variants. The degree of sequence identity cited herein is determined across the length of the sequence determined by the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular) using an affine gap search with the following parameters: gap open penalty 12, gap extension penalty 1.

The invention also provides nucleic acid including a fragment of one or more of the nucleotide sequences set out herein, including the NMB open reading frames shown in Appendix B hereto. The fragment should comprise at least n consecutive nucleotides from the sequences and, depending on the particular sequence, n is 10 or more (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 60, 75, 100 or more). Preferably, the fragment is unique to the genome of *N. meningitidis*, that is to say it is not present in the genome of another organism. More preferably, the fragment is unique to the genome of strain B of *N. meningitidis*. The invention also provides nucleic acid that hybridizes to those provided herein. Conditions for hybridizing are disclosed herein.

The invention also provides nucleic acid including sequences complementary to those described above (e.g., for antisense, for probes, or for amplification primers).

Nucleic acid according to the invention can, of course, be prepared in many ways (e.g., by chemical synthesis, from DNA libraries, from the organism itself, etc.) and can take various forms (e.g., single-stranded, double-stranded, vectors, probes, primers, etc.). The term "nucleic acid" includes DNA and RNA, and also their analogs, such as those containing modified backbones, and also peptide nucleic acid (PNA) etc.

It will be appreciated that, as SEQ ID NOs:1-961 of the '573 application represent the substantially complete genome of the organism, with partial overlap, references to SEQ ID NOs:1-961 of the '573 application include within their scope references to the complete genomic sequence, that is, SEQ ID NO. 1 hereof. For example, where two SEQ ID NOs overlap, the invention encompasses the single sequence which is formed by assembling the two overlapping sequences, which full sequence will be found in SEQ ID NO. 1 hereof. Thus, for instance, a nucleotide sequence which bridges two SEQ ID NOs but is not present in its entirety in either SEQ ID NO is still within the scope of the invention. Such a sequence will be present in its entirety in the single full length sequence of SEQ ID NO. 1 of the present application.

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The invention also provides vectors including nucleotide sequences of the invention (e.g., expression vectors, sequencing vectors, cloning vectors, etc.) and host cells transformed with such vectors.

According to a further aspect, the invention provides a protein including an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out herein. It also provides proteins comprising sequences having sequence identity to those proteins. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). Sequence identity is determined as above disclosed. These homologous proteins include mutants and allelic variants, encoded within the *N. meningitidis* nucleotide sequence set out herein.

The invention further provides proteins including fragments of an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out in the sequence listing. The fragments should comprise at least *n* consecutive amino acids from the sequences and, depending on the particular sequence, *n* is 7 or more (e.g., 8, 10, 12, 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

The proteins of the invention can, of course, be prepared by various means (e.g., recombinant expression, purification from cell culture, chemical synthesis, etc.) and in various forms (e.g. native, fusions etc.). They are preferably prepared in substantially isolated form (i.e., substantially free from other *N. meningitidis* host cell proteins).

Various tests can be used to assess the *in vivo* immunogenicity of the proteins of the invention. For example, the proteins can be expressed recombinantly or chemically synthesized and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question; i.e., the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The invention also provides nucleic acid encoding a protein of the invention.

In a further aspect, the invention provides a computer, a computer memory, a computer storage medium (e.g., floppy disk, fixed disk, CD-ROM, etc.), and/or a computer database containing the nucleotide sequence of nucleic acid according to the invention. Preferably, it contains one or more of the *N. meningitidis* nucleotide sequences set out herein.

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This may be used in the analysis of the *N. meningitidis* nucleotide sequences set out herein. For instance, it may be used in a search to identify open reading frames (ORFs) or coding sequences within the sequences.

In a further aspect, the invention provides a method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within a *N. meningitidis* nucleotide sequence set out herein. Similarly, the invention provides the use of a *N. meningitidis* nucleotide sequence set out herein in a search for putative open reading frames or protein-coding sequences.

Open-reading frame or protein-coding sequence analysis is generally performed on a computer using standard bioinformatic techniques. Typical algorithms or program used in the analysis include ORFFINDER (NCBI), GENMARK [Borodovsky & McIninch (1993) *Computers Chem* 17:122-133], and GLIMMER [Salzberg et al. (1998) *Nucl Acids Res* 26:544-548].

A search for an open reading frame or protein-coding sequence may comprise the steps of searching a *N. meningitidis* nucleotide sequence set out herein for an initiation codon and searching the upstream sequence for an in-frame termination codon. The intervening codons represent a putative protein-coding sequence. Typically, all six possible reading frames of a sequence will be searched.

An amino acid sequence identified in this way can be expressed using any suitable system to give a protein. This protein can be used to raise antibodies which recognize epitopes within the identified amino acid sequence. These antibodies can be used to screen *N. meningitidis* to detect the presence of a protein comprising the identified amino acid sequence.

Furthermore, once an ORF or protein-coding sequence is identified, the sequence can be compared with sequence databases. Sequence analysis tools can be found at NCBI (<http://www.ncbi.nlm.nih.gov>) e.g., the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Suitable databases for comparison include the nonredundant GenBank, EMBL, DDBJ and PDB sequences, and the nonredundant GenBank CDS translations, PDB,

SwissProt, Spupdate and PIR sequences. This comparison may give an indication of the function of a protein.

Hydrophobic domains in an amino acid sequence can be predicted using algorithms such as those based on the statistical studies of Esposti *et al.* [Critical evaluation of the hydropathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. Hydrophobic domains represent potential transmembrane regions or hydrophobic leader sequences, which suggest that the proteins may be secreted or be surface-located. These properties are typically representative of good immunogens.

Similarly, transmembrane domains or leader sequences can be predicted using the PSORT algorithm (<http://www.psort.nibb.ac.jp>), and functional domains can be predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

The invention also provides nucleic acid including an open reading frame or protein-coding sequence present in a *N. meningitidis* nucleotide sequence set out herein. Furthermore, the invention provides a protein including the amino acid sequence encoded by this open reading frame or protein-coding sequence.

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means known to those skilled in the art.

The antibodies of the invention can be used in a variety of ways, e.g., for confirmation that a protein is expressed, or to confirm where a protein is expressed. Labeled antibody (e.g., fluorescent labeling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein, for instance.

According to a further aspect, the invention provides compositions including protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, as immunogenic compositions, or as diagnostic reagents.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (e.g., as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of (i) a medicament for treating or preventing infection due to Neisserial bacteria (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria. Said Neisserial bacteria may be any species or

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strain (such as *N. gonorrhoeae*) but are preferably *N. meningitidis*, especially strain A, strain B or strain C.

In still yet another aspect, the present invention provides for compositions including proteins, nucleic acid molecules, or antibodies. More preferable aspects of the present invention are drawn to immunogenic compositions of proteins. Further preferable aspects of the present invention contemplate pharmaceutical immunogenic compositions of proteins or vaccines and the use thereof in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria, preferably infection of MenB.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression. A process which may further include chemical synthesis of proteins and/or chemical synthesis (at least in part) of nucleotides.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Another aspect of the present invention provides for a process for detecting antibodies that selectably bind to antigens or polypeptides or proteins specific to any species or strain of Neisserial bacteria and preferably to strains of *N. gonorrhoeae* but more preferably to strains of *N. meningitidis*, especially strain A, strain B or strain C, more preferably MenB, where the process comprises the steps of: (a) contacting antigen or polypeptide or protein according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

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Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Methodology - Summary of standard procedures and techniques.

General

This invention provides *Neisseria meningitidis* MenB nucleotide sequences, amino acid sequences encoded therein. With these disclosed sequences, nucleic acid probe assays and expression cassettes and vectors can be produced. The proteins can also be chemically synthesized. The expression vectors can be transformed into host cells to produce proteins. The purified or isolated polypeptides can be used to produce antibodies to detect MenB proteins. Also, the host cells or extracts can be utilized for biological assays to isolate agonists or antagonists. In addition, with these sequences one can search to identify open reading frames and identify amino acid sequences. The proteins may also be used in immunogenic compositions and as vaccine components.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature e.g., Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and ii* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C.C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

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All publications, patents, and patent applications cited herein are incorporated in full by reference.

Expression systems

The *Neisseria* MenB nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, plant cells, baculoviruses, bacteria, and yeast.

i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g., structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation (Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual*, 2nd ed.).

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible). Depending on the promoter selected, many promoters may be inducible using known substrates, such as the use of the mouse mammary tumor virus (MMTV) promoter with the glucocorticoid responsive element (GRE) that is induced by glucocorticoid in hormone-responsive transformed cells (see for example, U.S. Patent 5,783,681).

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The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.). Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer (Dijkema et al (1985) *EMBO J.* 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) *Proc. Natl. Acad. Sci.* 79:6777) and from human cytomegalovirus (Boshart et al. (1985) *Cell* 41:521). Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237).

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the

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mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation (Birnstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci.* 14:105). These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 (Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*).

Usually, the above-described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 (Gluzman (1981) *Cell* 23:175) or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replication systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 (Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946) and pHEBO (Shimizu et al. (1986) *Mol. Cell. Biol.* 6:1074).

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection

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(ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines.

ii. Plant Cellular Expression Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: U.S. 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wirsal et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, Gibberellins: in: *Advanced Plant Physiology*,. Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gene is not

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readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Repr.*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

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Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet.*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*,

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Ranunculus, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the

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homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (e.g., structural gene)

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into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlaskovits et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human (alpha) α -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion

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protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μm in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of

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recombinant virus) of occlusion bodies. *Current Protocols in Microbiology* Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (PCT Pub. No. WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, *et al.* (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. *See*, e.g., Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, e.g., HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, e.g., proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) (Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173). Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) (Chang *et al.* (1977) *Nature* 198:1056), and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) (Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; U.S. Patent 4,738,921; EPO Publ. Nos. 036 776 and 121 775). The beta-lactamase (*bla*) promoter system (Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)), bacteriophage lambda PL (Shimatake *et al.* (1981) *Nature* 292:128) and T5 (U.S. Patent 4,689,406) promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter (U.S. Patent 4,551,433). For

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example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor (Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21). Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system (Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074). In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO Publ. No. 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine *et al.* (1975) *Nature* 254:34). The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' end of *E. coli* 16S rRNA (Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberg)). To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site, it is often necessary to optimize the distance between the SD sequence and the ATG of the eukaryotic gene (Sambrook *et al.* (1989) "Expression of cloned genes in *Escherichia coli*." In *Molecular Cloning: A Laboratory Manual*).

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* or *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO Publ. No. 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is

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fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene (Nagai *et al.* (1984) *Nature* 309:810). Fusion proteins can also be made with sequences from the *lacZ* (Jia *et al.* (1987) *Gene* 60:197), *trpE* (Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11), and *Chey* (EPO Publ. No. 324 647) genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated (Miller *et al.* (1989) *Bio/Technology* 7:698).

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria (U.S. Patent 4,336,336). The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) (Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghayeb *et al.* (1984) *EMBO J.* 3:2437) and the *E. coli* alkaline phosphatase signal sequence (*phoA*) (Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212). As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. No. 244 042).

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the

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coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EPO Publ. No. 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline (Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469). Selectable

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markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541), *Escherichia coli* (Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EPO Publ. Nos. 036 776, 136 829 and 136 907), *Streptococcus cremoris* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655); *Streptococcus lividans* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655), *Streptomyces lividans* (U.S. Patent 4,745,056).

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with CaCl_2 or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. (See e.g., use of *Bacillus*: Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541; use of *Campylobacter*: Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; and Wang *et al.* (1990) *J. Bacteriol.* 172:949; use of *Escherichia coli*: Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; use of *Lactobacillus*: Chassy *et al.* (1987) *FEMS Microbiol. Lett.* 44:173; use of *Pseudomonas*: Fiedler *et al.* (1988) *Anal. Biochem.* 170:38; use of *Staphylococcus*: Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203; use of *Streptococcus*: Barany *et al.* (1980) *J. Bacteriol.* 144:698;

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Harlander (1987) "Transformation of *Streptococcus lactis* by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* 1:412.

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EPO Publ. No. 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO Publ. No. 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences (Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1).

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (U.S. Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of

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either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EPO Publ. No. 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, (Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109;).

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, plant, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See e.g., EPO Publ. No. 196056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (e.g., WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can

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be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EPO Publ. No. 012 873; JPO Publ. No. 62:096,086) and the A-factor gene (U.S. Patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EPO Publ. No. 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (U.S. Patent Nos. 4,546,083 and 4,870,008; EPO Publ. No. 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alpha factor. (See e.g., PCT Publ. No. WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 (Botstein *et al.* (1979) *Gene* 8:17-24), pCl/1 (Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646), and YRp17 (Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157). In addition, a replicon may be

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either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See e.g., Brake *et al.*, *supra*.

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome (Orr-Weaver *et al.* (1983) *Methods in Enzymol.* 101:228-245). An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced (Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750). The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions (Butt *et al.* (1987) *Microbiol. Rev.* 51:351).

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker

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that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors and methods of introducing exogenous DNA into yeast hosts have been developed for, *inter alia*, the following yeasts: *Candida albicans* (Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142); *Candida maltosa* (Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141); *Hansenula polymorpha* (Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302); *Kluyveromyces fragilis* (Das, *et al.* (1984) *J. Bacteriol.* 158:1165); *Kluyveromyces lactis* (De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135); *Pichia guillermondii* (Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141); *Pichia pastoris* (Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; U.S. Patent Nos. 4,837,148 and 4,929,555); *Saccharomyces cerevisiae* (Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163); *Schizosaccharomyces pombe* (Beach and Nurse (1981) *Nature* 300:706); and *Yarrowia lipolytica* (Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49).

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See e.g., [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; *Candida*]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; *Hansenula*]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; *Kluyveromyces*]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; U.S. Patent Nos. 4,837,148 and 4,929,555; *Pichia*]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 *Saccharomyces*]; [Beach and Nurse (1981) *Nature* 300:706; *Schizosaccharomyces*]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; *Yarrowia*].

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Definitions

A composition containing X is “substantially free of” Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term “heterologous” refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell.

An “origin of replication” is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A “mutant” sequence is defined as a DNA, RNA or amino acid sequence differing from but having homology with the native or disclosed sequence. Depending on the particular sequence, the degree of homology between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more) which is calculated as described above. As used herein, an “allelic variant” of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs at essentially the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions. (see, for example, U.S. Patent 5,753,235).

Antibodies

As used herein, the term “antibody” refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An “antibody combining site” is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. “Antibody” includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanized antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying *Neisseria* MenB proteins. Antibodies elicited against the proteins of the present invention bind to antigenic polypeptides or proteins or protein fragments that are present and specifically associated with strains of *Neisseria meningitidis* MenB. In some instances, these antigens may be associated with specific strains, such as those antigens specific for the MenB strains. The antibodies of the invention may be immobilized to a matrix and utilized in an immunoassay or on an affinity chromatography column, to enable the detection and/or separation of polypeptides, proteins or protein fragments or cells comprising such polypeptides, proteins or protein fragments. Alternatively, such polypeptides, proteins or protein fragments may be immobilized so as to detect antibodies bindably specific thereto.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this

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invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein (*Nature* (1975) 256:495-96), or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells that express membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various

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labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I , or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Antigens, immunogens, polypeptides, proteins or protein fragments of the present invention elicit formation of specific binding partner antibodies. These antigens, immunogens, polypeptides, proteins or protein fragments of the present invention comprise immunogenic compositions of the present invention. Such immunogenic compositions may further comprise or include adjuvants, carriers, or other compositions that promote or enhance or stabilize the antigens, polypeptides, proteins or protein fragments of the present invention. Such adjuvants and carriers will be readily apparent to those of ordinary skill in the art.

Pharmaceutical Compositions

Pharmaceutical compositions can include either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature, when given to a patient that is febrile. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in

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advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

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Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal and transcutaneous applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

Vaccines according to the invention may either be prophylactic (i.e., to prevent infection) or therapeutic (i.e., to treat disease after infection).

Such vaccines comprise immunizing antigen(s) or immunogen(s), immunogenic polypeptide, protein(s) or protein fragments, or nucleic acids (e.g., ribonucleic acid or deoxyribonucleic acid), usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the immunogen or antigen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a

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larger particle size emulsion, and (c) RibiTM adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DetoxTM); (3) saponin adjuvants, such as StimulonTM (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.*; (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129; see, e.g., WO 93/13302 and WO 92/19265; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59 are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The vaccine compositions comprising immunogenic compositions (e.g., which may include the antigen, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Alternatively, vaccine compositions comprising immunogenic compositions may comprise an antigen, polypeptide, protein, protein fragment or nucleic acid in a pharmaceutically acceptable carrier.

More specifically, vaccines comprising immunogenic compositions comprise an immunologically effective amount of the immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of

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individual to be treated (e.g., nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Typically, the vaccine compositions or immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

The immunogenic compositions are conventionally administered parenterally, e.g., by injection, either subcutaneously or intramuscularly. Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal and transcutaneous applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed (e.g., Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648).

Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs, including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus,

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paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses e.g., MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (e.g., HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No.1 VR-590), Kirsten, Harvey Sarcoma Virus and

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Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native

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D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (i.e., there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors comprising sequences of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in U.S. Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578,

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WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, *Nature* 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example

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ATCC VR-925; Trinit virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed to transform a host cell. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved

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further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in U.S. 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, *Biochemistry*, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprise a therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, transdermally or transcutaneously, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule. See WO98/20734.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., WO93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Polynucleotide and Polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A. Polypeptides

One example are polypeptides which include, without limitation: asialoorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF),

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granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

B. Hormones, Vitamins, Etc.

Other groups that can be included in a pharmaceutical composition include, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, *etc.*

Also, polyalkylene glycol can be included in a pharmaceutical compositions with the desired polynucleotides and/or polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide) may be included in a pharmaceutical composition.

D. Lipids, and Liposomes

The desired polynucleotide or polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid or polypeptide. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

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Cationic liposomes are readily available. For example, N(1-2,3-dioleoyloxy)propyl)-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See e.g., Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348; Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E. Lipoproteins

In addition, lipoproteins can be included with the polynucleotide or polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These

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lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E; over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid sequences of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol. (supra)*; Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750.

Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443.

Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA.

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Further description of lipoproteins can be found in Zuckermann et al., PCT. Appln. No. US97/14465.

F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide and/or polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples of useful polypeptides include histones, protamines; human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as Φ X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

G. Synthetic Polycationic Agents

Synthetic polycationic agents which are useful in pharmaceutical compositions include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides or polypeptides.

Immunodiagnostic Assays

Neisseria MenB antigens, or antigenic fragments thereof, of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-*Neisseria* MenB antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to *Neisseria* MenB proteins or fragments thereof within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

Nucleic Acid Hybridization

“Hybridization” refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the

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stringency of the washing conditions following hybridization. See Sambrook *et al.* (*supra*) Volume 2, chapter 9, pages 9.47 to 9.57.

“Stringency” refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (T_m) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4\%(G + C) - 0.6\%(\text{formamide}) - 600/n - 1.5\%(\text{mismatch})$$

where C_i is the salt concentration (monovalent ions) and n is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138:267-284).

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In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (i.e., stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so

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a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Neisserial sequence (or its complement) -- some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* (*J. Am. Chem. Soc.* (1981) 103:3185), or according to Urdea *et al.* (*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461), or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated e.g., backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* (e.g., see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387); analogues such as peptide nucleic acids may also be

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used (e.g., see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386).

One example of a nucleotide hybridization assay is described by Urdea *et al.* in international patent application WO92/02526 (see also U.S. Patent 5,124,246).

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* (*Meth. Enzymol.* (1987) 155: 335-350); US patent 4,683,195; and US patent 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Neisserial sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labeled probe will hybridize to the Neisserial sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al.* (*supra*). mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labeled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labeled with a radioactive moiety.

EXAMPLES

The invention is based on the 961 nucleotide sequences from the genome of *N. meningitidis* set out in Appendix C, SEQ ID NOs:1-961 of the '573 application, which together represent substantially the complete genome of serotype B of *N. meningitidis*, as well as the full length genome sequence shown in Appendix D, SEQ ID NO 1068 of the '573

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application, and the full length genome sequence shown in Appendix A hereto, SEQ ID NO. 1.

It will be self-evident to the skilled person how this sequence information can be utilized according to the invention, as above described.

The standard techniques and procedures which may be employed in order to perform the invention (*e.g.* to utilize the disclosed sequences to predict polypeptides useful for vaccination or diagnostic purposes) were summarized above. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

These sequences are derived from contigs shown in Appendix C (SEQ ID NOs 1-961) and from the full length genome sequence shown in Appendix D (SEQ ID NO 1068), which were prepared during the sequencing of the genome of *N. meningitidis* (strain B). The full length sequence was assembled using the TIGR Assembler as described by G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995) [*see also* R. D. Fleischmann, et al., Science 269, 496-512 (1995); C. M. Fraser, et al., Science 270, 397-403 (1995); C. J. Bult, et al., Science 273, 1058-73 (1996); C. M. Fraser, et. al, Nature 390, 580-586 (1997); J.-F. Tomb, et. al., Nature 388, 539-547 (1997); H. P. Klenk, et al., Nature 390, 364-70 (1997); C. M. Fraser, et al., Science 281, 375-88 (1998); M. J. Gardner, et al., Science 282, 1126-1132 (1998); K. E. Nelson, et al., Nature 399, 323-9 (1999)]. Then, using the above-described methods, putative translation products of the sequences were determined. Computer analysis of the translation products were determined based on database comparisons. Corresponding gene and protein sequences, if any, were identified in *Neisseria meningitidis* (Strain A) and *Neisseria gonorrhoeae*. Then the proteins were expressed, purified, and characterized to assess their antigenicity and immunogenicity.

In particular, the following methods were used to express, purify, and biochemically characterize the proteins of the invention.

Chromosomal DNA Preparation

N. meningitidis strain 2996 was grown to exponential phase in 100 ml of GC medium, harvested by centrifugation, and resuspended in 5 ml buffer (20% Sucrose, 50 mM Tris-HCl, 50 mM EDTA, adjusted to pH 8.0). After 10 minutes incubation on ice, the bacteria were

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lysed by adding 10 ml lysis solution (50 mM NaCl, 1% Na-Sarkosyl, 50 µg/ml Proteinase K), and the suspension was incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one CHCl_3 /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70% ethanol and redissolved in 4 ml buffer (10 mM Tris-HCl, 1mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

For most ORFs, the 5' primers included two restriction enzyme recognition sites (*Bam*HI-*Nde*I, *Bam*HI-*Nhe*I, or *Eco*RI-*Nhe*I, depending on the gene's restriction pattern); the 3' primers included a *Xho*I restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *Bam*HI-*Xho*I or *Eco*RI-*Xho*I), and pET21b+ (using either *Nde*I-*Xho*I or *Nhe*I-*Xho*I).

5'-end primer tail:	<u>CGCGGATCCCATATG</u>	(<i>Bam</i> HI- <i>Nde</i> I)
	<u>CGCGGATCCGCTAGC</u>	(<i>Bam</i> HI- <i>Nhe</i> I)
	<u>CCGGAATTCTAGCTAGC</u>	(<i>Eco</i> RI- <i>Nhe</i> I)
3'-end primer tail:	<u>CCCGCTCGAG</u>	(<i>Xho</i> I)

For some ORFs, two different amplifications were performed to clone each ORF in the two expression systems. Two different 5' primers were used for each ORF; the same 3' *Xho*I primer was used as before:

5'-end primer tail:	<u>GGAATTCCATATGGCCATGG</u>	(<i>Nde</i> I)
5'-end primer tail:	<u>CGGGATCC</u>	(<i>Bam</i> HI)

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Other ORFs were cloned in the pTRC expression vector and expressed as an amino-terminus His-tag fusion. The predicted signal peptide may be included in the final product. *NheI*-*BamHI* restriction sites were incorporated using primers:

5'-end primer tail: GATCAGCTAGCCATATG (*NheI*)

3'-end primer tail: CGGGATCC (*BamHI*)

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$$T_m = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_m = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2 ml NH₄-OH, and deprotected by 5 hours incubation at 56 °C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100µl or 1ml of water. OD₂₆₀ was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10 pmol/µl.

Table 1 shows the forward and reverse primers used for each amplification. In certain cases, it might be noted that the sequence of the primer does not exactly match the sequence in the ORF. When initial amplifications are performed, the complete 5' and/or 3' sequence may not be known for some meningococcal ORFs, although the corresponding sequences may have been identified in gonococcus. For amplification, the gonococcal sequences could thus be used as the basis for primer design, altered to take account of codon preference. In particular, the following codons may be changed: ATA→ATT; TCG→TCT; CAG→CAA; AAG→AAA; GAG→GAA; CGA and CGG→CGC; GGG→GGC.

Amplification

The standard PCR protocol was as follows: 50-200 ng of genomic DNA were used as a template in the presence of 20-40 µM of each oligo, 400-800 µM dNTPs solution, 1x PCR

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buffer (including 1.5 mM MgCl₂), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

In some cases, PCR was optimised by the addition of 10µl DMSO or 50 µl 2M betaine.

After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at 95°C), each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridization temperature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at 72°C.

The standard cycles were as follows:

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds 95°C	30 seconds 50-55°C	30-60 seconds 72°C
Last 30 cycles	30 seconds 95°C	30 seconds 65-70°C	30-60 seconds 72°C

The elongation time varied according to the length of the ORF to be amplified.

The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, 1/10 of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30µl or 50µl of either water or 10mM Tris, pH 8.5.

Digestion of PCR fragments

The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

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NdeI/XhoI or *NheI/XhoI* for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion

BamHI/XhoI or *EcoRI/XhoI* for cloning into pGEX-KG and further expression of the protein as a GST N-terminus fusion.

For ORF 76, *NheI/BamHI* for cloning into pTRC-HisA vector and further expression of the protein as N-terminus His-tag fusion.

Each purified DNA fragment was incubated (37°C for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs) in a either 30 or 40 µl final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 (or 50) µl of either water or 10mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

Digestion of the cloning vectors (pET22B, pGEX-KG and pTRC-His A)

10 µg plasmid was double-digested with 50 units of each restriction enzyme in 200 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37°C. After loading the whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick Gel Extraction Kit and the DNA was eluted in 50 µl of 10 mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD₂₆₀ of the sample, and adjusted to 50 µg/µl. 1 µl of plasmid was used for each cloning procedure.

Cloning

The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20 µl, a molar ratio of 3:1 fragment/vector was ligated using 0.5 µl of NEB T4 DNA ligase (400 units/µl), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

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In order to introduce the recombinant plasmid in a suitable strain, 100 µl *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37°C for 3 minutes, then, after adding 800 µl LB broth, again at 37°C for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200 µl of the supernatant. The suspension was then plated on LB ampicillin (100 mg/ml).

The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37 °C in either 2 ml (pGEX or pTC clones) or 5ml (pET clones) LB broth + 100 µg/ml ampicillin. The cells were then pelleted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30 µl. 5 µl of each individual miniprep (approximately 1g) were digested with either *NdeI/XhoI* or *BamHI/XhoI* and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.

Cloning

Certain ORFs may be cloned into the pGEX-HIS vector using *EcoRI-PstI*, *EcoRI-SalI*, or *SalI-PstI* cloning sites. After cloning, the recombinant plasmids may be introduced in the *E.coli* host W3110.

Expression

Each ORF cloned into the expression vector may then be transformed into the strain suitable for expression of the recombinant protein product. 1 µl of each construct was used to transform 30 µl of *E.coli* BL21 (pGEX vector), *E.coli* TOP 10 (pTRC vector) or *E.coli* BL21-DE3 (pET vector), as described above. In the case of the pGEX-His vector, the same *E.coli* strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2ml LB+Amp (100 µg/ml), incubated at 37°C overnight, then diluted 1:30 in 20 ml of LB+Amp (100 µg/ml) in 100 ml flasks, making sure that the OD₆₀₀ ranged between 0.1 and 0.15. The flasks were incubated at 30°C into gyratory water bath shakers until OD indicated exponential growth suitable for induction of expression (0.4-0.8 OD for

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pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2 mM. After 3 hours incubation at 30°C, the final concentration of the sample was checked by OD. In order to check expression, 1ml of each sample was removed, centrifuged in a microfuge, the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000g and the pellet resuspended in PBS for further use.

GST-fusion proteins large-scale purification.

A single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600 ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD₅₅₀ 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000 rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Glutathione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD₂₈₀ of 0.02-0.06. The GST-fusion protein was eluted by addition of 700µl cold Glutathione elution buffer (10mM reduced glutathione, 50mM Tris-HCl) and fractions collected until the OD₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M'') (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

His-fusion soluble proteins large-scale purification.

A single colony was grown overnight at 37°C on a LB + Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600ml fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000 rpm at 4°C, the supernatant was discarded and the bacterial pellet was resuspended in 7.5ml cold 10mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Ni²⁺-resin (Pharmacia) (previously washed with 10mM imidazole buffer) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold 10mM imidazole buffer for 10 minutes, resuspended in 1ml cold 10mM imidazole buffer and loaded on a disposable column. The resin was washed at 4°C with 2ml cold 10mM imidazole buffer until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The resin was washed with 2ml cold 20mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8) until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl cold 250mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 250 mM imidazole, pH 8) and fractions collected until the O.D₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

His-fusion insoluble proteins large-scale purification.

A single colony was grown overnight at 37 °C on a LB + Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600ml fresh medium and let to grow at the optimal temperature (37°C) to O.D₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 8.8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen

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and thawed twice and centrifuged again. The supernatant was stored at -20°C, while the pellets were resuspended in 2 ml guanidine buffer (6M guanidine hydrochloride, 100mM phosphate buffer, 10 mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000 rpm for 40 minutes. The supernatant was mixed with 150µl Ni²⁺-resin (Pharmacia) (previously washed with buffer B) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700 g for 5 minutes at 4°C. The resin was washed twice with 10 ml buffer B for 10 minutes, resuspended in 1ml buffer B, and loaded on a disposable column. The resin was washed at room temperature with 2ml buffer B until the flow-through reached the OD₂₈₀ of 0.02-0.06. The resin was washed with 2ml buffer C (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl elution buffer (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the OD₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

His-fusion proteins renaturation

10% glycerol was added to the denatured proteins. The proteins were then diluted to 20µg/ml using dialysis buffer I (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4°C for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was evaluated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times \text{OD}_{280}) - (0.76 \times \text{OD}_{260})$$

Mice immunisations

20µg of each purified protein were used to immunise mice intraperitoneally. In the case of some ORFs, Balb-C mice were immunised with Al(OH)₃ as adjuvant on days 1, 21 and 42, and immune response was monitored in samples taken on day 56. For other ORFs, CD1 mice could be immunised using the same protocol. For other ORFs, CD1 mice could be immunised using Freund's adjuvant, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for still other

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ORFs, CD1 mice could be immunised with Freund's adjuvant, but the immune response was measured on day 49.

ELISA assay (sera analysis)

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200 µl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200 µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN₃ in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100 µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100 µl of substrate buffer for HRP (25 ml of citrate buffer pH5, 10 mg of O-phenildiamine and 10 µl of H₂O) were added to each well and the plates were left at room temperature for 20 minutes. 100 µl H₂SO₄ was added to each well and OD₄₉₀ was followed. The ELISA was considered positive when OD490 was 2.5 times the respective pre-immune sera.

FACScan bacteria Binding Assay procedure.

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following

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OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000 rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN₃) and centrifuged for 5 minutes at 4000 rpm. Cells were resuspended in blocking buffer to reach OD₆₂₀ of 0.07. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:200) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000 rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)₂ goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H Treshold:92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539. Compensation values: 0.

OMV preparations

Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes and the bacteria disrupted by sonication for 10' on ice (50% duty cycle, 50% output). Unbroken cells were removed by centrifugation at 5000g for 10 minutes and the total cell envelope fraction recovered by centrifugation at 50000g at 4°C for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000g for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

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Whole Extracts preparation

Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30' minutes.

Western blotting

Purified proteins (500ng/lane), outer membrane vesicles (5 µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, in transferring buffer (0.3 % Tris base, 1.44 % glycine, 20% methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with 1:200 mice sera diluted in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labeled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

Bactericidal assay

MC58 strain was grown overnight at 37°C on chocolate agar plates. 5-7 colonies were collected and used to inoculate 7ml Mueller-Hinton broth. The suspension was incubated at 37°C on a nutator and let to grow until OD₆₂₀ was in between 0.5-0.8. The culture was aliquoted into sterile 1.5ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD₆₂₀ of 0.5, diluted 1:20000 in Gey's buffer and stored at 25°C.

50µl of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25µl of diluted (1:100) mice sera (dilution buffer: Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4°C. 25µl of the previously described bacterial suspension were added to each well. 25µl of either heat-inactivated (56°C waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22µl of each sample/well were plated on Mueller-

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Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37°C with rotation and then 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1h were counted.

The following DNA and amino acid sequences are identified by titles of the following form: [g, m, or a] [#].[seq or pep], where "g" means a sequence from *N. gonorrhoeae*, "m" means a sequence from *N. meningitidis B*, and "a" means a sequence from *N. meningitidis A*; "#" means the number of the sequence; "seq" means a DNA sequence, and "pep" means an amino acid sequence. For example, "g001.seq" refers to an *N. gonorrhoeae* DNA sequence, number 1. The presence of the suffix "-1" or "-2" to these sequences indicates an additional sequence found for the same ORF. Further, open reading frames are identified as ORF #, where "#" means the number of the ORF, corresponding to the number of the sequence which encodes the ORF, and the ORF designations may be suffixed with ".ng" or ".a", indicating that the ORF corresponds to a *N. gonorrhoeae* sequence or a *N. meningitidis A* sequence, respectively. Computer analysis was performed for the comparisons that follow between "g", "m", and "a" peptide sequences; and therein the "pep" suffix is implied where not expressly stated.

EXAMPLE 1

The following ORFs were predicted from the contig sequences and/or the full length sequences using the methods herein described.

Localization of the ORFs

ORF: contig:

279 gnm4.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 2>:

m279.seq

```

1  ATAACGCGGA TTTGCGGCTG CTTGATTTCA ACGGTTTTCA GGGCTTCGGC
51  AAGTTTGTCT GCGGCGGGTT TCATCAGGCT GCAATGGGAA GGTACGGACA
101 CGGGCAGCGG CAGGGCGCGT TTGGCACCAG CTCTTTTGGC GGCAGCCATG
151 GCGCGTCCGA CGGCGGCGGC GTTGCTTGCA ATCACGATTT GTCCGGGTGA
201 GTTGAAGTTG ACGGCTTCGA CCACTTCGCT TTGGGCGGCT TCGGCACAAA
251 TGGCTTTAAC CTGCTCATCT TCCAAGCCGA GAATCGCCGC CATGCGCCCC
301 ACGCCTTGCG GTACGGCGGA CTGCATCAGT TCGGCGCGCA GGCACACGAG
351 TTTGACCGCG TCGGCAAAAT TCAATGCGCC GCGGCAACG AGTGCGGTGT
401 ATTCGCCGAG GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCGCT
451 TCTAAATAG
```

```

1  ITRICGCLIS TVFRASASLS AAGFIRLQWE GTDTGSGRAR LAPASLAAAM
51  ARPTAAALPA ITICPGELKL TASTTSLWAA SAQMALTCSS SKPRIAAIAP
101 TPCGTADCIS SARRRTSLTA SAKFNAPAAAT SAVYSPRLCP ATAAGVLPAP
151 SK*

```

g279.req

1	atgacgcgga	tttgcggctg	cttgatttca	acggttttga	gtgttttcggc
51	aagtttgtcg	gcggcggggt	tcatacaggct	gcaatgggaa	ggaacggata
101	ccggcagcgg	cagggcgcg	ttggctccgg	cttctttggc	ggcagccatg
151	gtgcgtccga	cggcgggcgg	gttgcttcca	atcacgactt	gtccggggcga
201	gttgaagtgt	acggcttcga	ccacttcgcc	ctgtgcggat	tgggcacaaa
251	tctgcctgac	ctgttcatct	tccaaaccca	aaatggccgc	cattgcgctt
301	acgccttgcg	gtacggcgga	ctgcatcagt	tggcgcgca	ggcggacgag
351	tttgcagcga	tcggcaaaa	ccaatgcttc	ggcggcgaca	agcgcggtgt
401	attcgcggag	gctgtgtccg	gcaacggcgg	caggcgtttt	gccgccact
451	tccaaataa				

g279.ppe

```

1  MTRICGCLIS TVLSVSASLS AAGFIRLQWE GTDTGSGRRAR LAPASLAAAM
51  VRPTAAALPA ITTCPGELKL TASTTSPCAD SAQICLTCSS SKPKMAAIAP
101 TPCGTADCIS SARRRTSLTA SAKSNSAAT SAVYSPRLCP ATAAGVLPPT
151 SK*

```

```

      10      20      30      40      50      60
m279.pep  ITRICGCLISTVFRASASLSAAGFIRLQWEGTDTGSGRARLAPASLAAAMARPTAAALPA
          :|||||: :|||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
g279      MTRICGCLISTVLSVSASLSAAGFIRLQWEGTDTGSGRARLAPASLAAAMVRPTAAALPA
          10      20      30      40      50      60

      70      80      90      100     110     120
m279.pep  ITICPGELKLTASTTSLWAASAQMALTCSSSKPRIAAIAPTTCGTADCISSARRRTSLTA
          || |||||: |||: |||: |||: |||: |||: |||: |||: |||: |||: |||:
g279      ITTCPGELKLTASTTSPCADSAQICLTCSSSKPKMAAIAPTTCGTADCISSARRRTSLTA
          70      80      90      100     110     120

      130     140     150
m279.pep  SAKFNAPAATSAVYSPRLCPATAAGVLPPASKX
          ||| || |||||: |||: |||: |||: |||: |||: |||: |||: |||: |||:
g279      SAKSNASAATSAVYSPRLCPATAAGVLPTTSKX
          130     140     150

```

a279.seq

1	ATGACNCNGA	TTTGCGGCTG	CTTGATTTC	ACGGTTTNN	GGGCTTCGGC
51	GAGTTTGTG	GCGGCGGGT	TCATGAGGT	GCAATGGGA	GGTACNGACA
101	CNGGCGAGC	CAGGCGCGT	TGGCGCCGG	CTTCTTTGGC	GGCAAGCATA
151	CGCGCGTCG	CGGCGGCGC	ATTGCTGCA	ATCAGCACT	TCCGGGCGA
201	GTTGAAGTT	ACGGCTTCA	CCACTTCAT	CTGTGCGGAT	TCCGGCGAAA
251	TTTGTTTTAC	CTGTTTCAT	TCCAAGCCG	GAATCGCCG	CATTGCGCCG
301	ACGCCTTGCG	GTACGGCGGA	CTGCATCAG	TCCGGCGCGA	CGCGCAGCAG
351	TTTGACCGCG	TCCGGAAAA	CCAATTGCGC	GGCGGCAACN	AGTCGGGTGT

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401 ATTCGCCGAN GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCCGCT
451 TCCGAATAG

This corresponds to the amino acid sequence <SEQ ID 7; ORF 279.a>:

a279.pep
1 MTXICGCLIS TVXRSASLS AAGFMRLQWE GTDTGSGRAR LAPASLAASI
51 ARSTAAALPA ITTCPGELKL TASTTSSCAD SAQICFTCSS SKPRIAAIAP
101 TPCGTADCIS SARXRTSLTA SAKSNAPAAAT SAVYSPXLCP ATAAGVLPPA
151 SE*

m279/a279 ORFs 279 and 279.a showed a 88.2% identity in 152 aa overlap

	10	20	30	40	50	60
m279.pep	ITRICGLISTVFRASASLSAAGFIRLQWEGTDTGSGRARLAPASLAAAMARPTAAALPA					
	:					
a279	MTXICGCLISTVXRSASLSAAGFMRLQWEGTDTGSGRARLAPASLAASIARSTAAALPA					
	10	20	30	40	50	60
	70	80	90	100	110	120
m279.pep	ITICPGELKLTASTTSLWAASAQMALTCSSSKPRIAAIAPTPCGTADCISSARRRTSLTA					
a279	ITTCPGELKLTASTTSSCADSAQICFTCSSSKPRIAAIAPTPCGTADCISSARXRTSLTA					
	70	80	90	100	110	120
	130	140	150			
m279.pep	SAKFNAPAATSAVYSPRLCPATAAGVLPPASKX					
a279	SAKSNAPAATSAVYSPXLCPATAAGVLPPASEX					
	130	140	150			

519 and 519-1 gnm7.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 8>:

m519.seq (partial)
1 ..TCCGTTATCG GCGGTATGGA GTTGGACAAA ACGTTTGAAG AACGCGACGA
51 AATCAACAGT ACTGTTGTTG CGGCTTTGGA CGAGGCGGCC GGGgCTTgGG
101 GTGTGAAGGT TTTGCGTTAT GAGATTAAAG ACTTGTTCC GCCGCAAGAA
151 ATCCTTCGCT CAATGCAGGC GCAAATTACT GCCGAACGCG AAAAACGCGC
201 CCGTATCGCC GAATCCGAAG GTCGTAAAAT CGAACAAATC AACCTTGCCA
251 GTGGTCAGCG CGAAGCCGAA ATCCAACAAT CCGAAGGCGA GGCTCAGGCT
301 GCGGTCAATG CGTCAAATGC CGAGAAAATC GCCCGCATCA ACCGCGCCAA
351 AGGTGAAGCG GAATCCTTGC GCCTTGTGTC CGAAGCCAAT GCCGAAGCCA
401 TCCGTCAAAT TGCCGCCGCC CTTCAAACCC AAGGCGGTGC GGATGCGGTC
451 AATCTGAAGA TTGCGGAACA ATACGTCGCT GCGTTCAACA ATCTTGCCAA
501 AGAAAGCAAT ACGCTGATTA TGCCCGCCAA TGTGCGGAC ATCGGCAGCC
551 TGATTTCTGC CGGTATGAAA ATTATCGACA GCAGCAAAAC CGCCAAaTAA

This corresponds to the amino acid sequence <SEQ ID 9; ORF 519>:

m519.pep (partial)
1 ..SVIGRMELDK TFEERDEINS TVVAALDEAA GAWGVKVLRY EIKDLVPPQE
51 ILRSMQAQIT AEREKRARIA ESEGRKIEQI NLASGQREAE IQQSEGEAQA
101 AVNASNAEKI ARINRAKGEA ESLRLVAEAN AEAIRQIAAA LQTQGGADAV
151 NLKIAEQYVA AFNNLAKESN TLIMPANVAD IGSLISAGMK IIDSSKTAK*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 10>:

g519.seq
1 atggaatttt tcattatctt gttggcagcc gtcgccgttt tcggcttcaa
51 atcctttgtc gtcattcccc agcaggaagt ccacgttgtc gaaaggctcg

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```

101 ggcgtttcca tcgcgcctcg acggccggtt tgaatatttt gattcccttt
151 atcgaccgcg tcgcctaccg ccattcgctg aaagaaatcc ctttagacgt
201 acccagccag gtctgcatca cgcgcgataa tacgcaattg actggtgacg
251 gcatcatcta tttccaagta accgatccca aactcgcctc atacggttcg
301 agcaactaca ttatggcaat taccagctt gcccaaacga cgctgcgttc
351 cgttatcggg cgtatggagt tggacaaaac gtttgaagaa cgcgacgaaa
401 tcaacagtac cgtcgtctcc gccctcgatg aagccgccgg ggcttggggg
451 gtgaaagtcc tccgttacga aatcaaggat ttggttccgc cgcaagaaat
501 ctttcgcgca atgcaggcac aaattaccgc cgaacgcgaa aaacgcgccc
551 gtattgccga atccgaaggc cgtaaaatcg aacaaatcaa ctttgccagt
601 ggtcagcgtg aagccgaaat ccaacaatcc gaaggcgagg ctgaggctgc
651 ggtcaatgcg tccaatgccg agaaaatcgc ccgcatcaac cgcgccaaag
701 gcgaagcgga atccctgcgc cttggtgccc aagccaatgc cgaagccaac
751 cgtcaaattg ccgccgccct tcaaacccaa agcggggcgg atgcggtcaa
801 tctgaagatt gcgggacaat acgttaccgc gttcaaaaat cttgccaaag
851 aagacaatac gcggattaag cccgccaaag ttgccgaaat cgggaaccct
901 aattttcggc ggcatgaaaa attttcgcca gaagcaaaaa cggccaaata
951 a

```

This corresponds to the amino acid sequence <SEQ ID 11; ORF 519.ng>:

```

g519.pep
  1 MEFFIILLAA VAVFGFSFV VIPQQEVHVV ERLGRFHRAL TAGLNILIPF
 51 IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEEAQAANA SNAEKIARIN RAKGEAESLR LVAEANAAN
251 RQIAAALQTQ SGADAVNLKI AGQYVTAFKN LAKEDNTRIK PAKVAEIGNP
301 NFRRHEKFSP EAKTAK*

```

ORF 519 shows 87.5% identity over a 200 aa overlap with a predicted ORF (ORF 519.ng) from *N. gonorrhoeae*:

```

m519/g519

m519.pep
                                     10      20      30
SVIGRMELDKTFEERDEINSTVVAALDEAA
|||||

g519
YFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIGRMELDKTFEERDEINSTVVSALDEAA
  90      100      110      120      130      140

m519.pep
          40      50      60      70      80      90
GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
|||||

g519
GAWGVKVLRYEIKDLVPPQEILRAMAQITAEREKRARIAESEGRKIEQINLASGQREAE
  150      160      170      180      190      200

m519.pep
          100      110      120      130      140      150
IQQSEGEAQAANASNAEKIARINRAKGEAESLRLVAEANAIAIRQIAAALQTQGGADAV
|||||

g519
IQQSEGEAQAANASNAEKIARINRAKGEAESLRLVAEANAIAANRQIAAALQTQSGADAV
  210      220      230      240      250      260

m519.pep
          160      170      180      190      200
NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL-ISAGMKIIDSSKTAK
|||||

g519
NLKIAEQYVTAFKNLAKEDNTRIKPAKVAEIGNPNFRRHEKFSP EAKTAK
  270      280      290      300      310

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 12>:

a519.seq

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```

1  ATGGAATTTT TCATTATCTT GCTGGCAGCC GTCGTTGTTT TCGGCTTCAA
51  ATCCTTTGTT GTCATCCAC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GCGGTTTCCA TCGCGCCCTG ACGGCCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGGACAA TACGCAGCTG ACTGTTGACG
251 GTATCATCTA TTTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGGTTTCG
301 AGCAACTACA TTATGGCGAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAAT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGCAC CGTCGCTCC GCCCTCGATG AAGCCGCCGG AGCTTGGGGT
451 GTGAAGGTTT TGCATTATGA GATTAAAGAC TTGGTTCCGC CGCAAGAAAT
501 CTTTCGCTCA ATGCAGCGC AAATTACTGC TGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAATCG AACAAATCAA CCTTGCCAGT
601 GGTGAGCGCG AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCAAAATGCC AGAAAATCG CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCCGCCCT TCAAACCCAA GGCGGTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTCGCCGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GTATGAAAAT TATCGACAGC AGCAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 13; ORF 519.a>:

```

a519.pep
1  MEFFIILLAA VVVFGEKSFV VIPQQEVHV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESE RQIEQINLAS
201 GQREAEIQQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAEANAELI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

m519/a519 ORFs 519 and 519.a showed a 99.5% identity in 199 aa overlap

```

m519.pep
10 20 30
SVIGRMELDKTFEERDEINSTVVAALDEAA
|||||:|||||
a519 YFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIGRMELDKTFEERDEINSTVVSALDEAA
90 100 110 120 130 140

40 50 60 70 80 90
m519.pep GAWGVKVLRYEIKDLVPPQEILRSMAQAITAEREKRARIAESEGRKIEQINLASGQREAE
|||||:|||||
a519 GAWGVKVLRYEIKDLVPPQEILRSMAQAITAEREKRARIAESEGRKIEQINLASGQREAE
150 160 170 180 190 200

100 110 120 130 140 150
m519.pep IQQSEGEAQAAVNASNAEKIARINRAKGEAESLRLVAEANAELAIRQIAAALQTQGGADAV
|||||:|||||
a519 IQQSEGEAQAAVNASNAEKIARINRAKGEAESLRLVAEANAELAIRQIAAALQTQGGADAV
210 220 230 240 250 260

160 170 180 190 200
m519.pep NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
|||||:|||||
a519 NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
270 280 290 300 310

```

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 14>:

m519-1.seq

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```

1  ATGGAATTTT TCATTATCTT GTTGGTAGCC GTCGCCGTTT TCGGTTTCAA
51  ATCCTTTGTT GTCATCCAC AACAGGAAGT CCACGTTGTC GAAAGGCTGG
101 GCGGTTTCCA TCGCGCCCTG ACGGcCGGTT TGAATATTTT GATTCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGGACAA TACGCAGCTG ACTGTTGACG
251 GCATCATCTA TTTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGGTTTCG
301 AGCAACTACA TTATGGCGAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAGT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC TGTGTTGCG GCTTTGGACG AGGCGGCCGG GGCTTGGGGT
451 GTGAAGGTTT TGCATTATGA GATTAAAGAC TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCTCA ATGCAGGCGC AAATTACTGC CGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAAATCG AACAAATCAA CCTTGCCAGT
601 GGTCAGCGCG AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCATATGCG TCAATGCGG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCCGCCCT TCAAACCCAA GGCGGTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTCGCTGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GTATGAAAAT TATCGACAGC AGCAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 15; ORF 519-1>:

m519-1.

```

1  MEFFIILLVA VAVFGFKSFV VIPQQEVHVV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVA ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAEANAELAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 16>:

g519-1.seq

```

1  ATGGAATTTT TCATTATCTT GTTGGCAGCC GTCGCCGTTT TCGGCTTCAA
51  ATCCTTTGTC GTCATCCCCC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GCGGTTTCCA TCGCGCCCTG ACGGCCGTTT TGAATATTTT GATTCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGGATAA TACGCAATTG ACTGTTGACG
251 GCATCATCTA TTTCCAAGTA ACCGATCCCA AACTCGCCTC ATACGGTTTCG
301 AGCAACTACA TTATGGCAAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAGT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC CGTCGTCTCC GCCCTCGATG AAGCCGCCGG GGCTTGGGGT
451 GTGAAAGTCC TCCGTTACGA AATCAAGGAT TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCGCA ATGCAGGCAC AAATTACCGC CGAACGCGAA AAACGCGCCC
551 GTATTGCCGA ATCCGAAGGC CGTAAAATCG AACAAATCAA CCTTGCCAGT
601 GGTCAGCGTG AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCATATGCG TCCAATGCGG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GCGAAGCGGA ATCCCTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCCGCCCT TCAAACCCAA GGCGGGGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTAGCCGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GCATGAAAAT TATCGACAGC AGCAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 17; ORF 519-1.ng>:

g519-1.pep

```

1  MEFFIILLAA VAVFGFKSFV VIPQQEVHVV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAEANAELAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```


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m519-1/g519-1 ORFs 519-1 and 519-1.ng showed a 99.0% identity in 315 aa overlap

	10	20	30	40	50	60
g519-1.pep	MEFFIILLAAVAVFGFKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
	:					
m519-1	MEFFIILLVAVAVFGFKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
	10	20	30	40	50	60
g519-1.pep	70	80	90	100	110	120
	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
m519-1	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
	70	80	90	100	110	120
g519-1.pep	130	140	150	160	170	180
	RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRAMQAQITAERE					
m519-1	RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE					
	130	140	150	160	170	180
g519-1.pep	190	200	210	220	230	240
	KRARIAESEGRKIEQINLASQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR					
m519-1	KRARIAESEGRKIEQINLASQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR					
	190	200	210	220	230	240
g519-1.pep	250	260	270	280	290	300
	LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
m519-1	LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
	250	260	270	280	290	300
g519-1.pep	310					
	ISAGMKIIDSSKTAKX					
m519-1	ISAGMKIIDSSKTAKX					
	310					

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 18>:

a519-1.seq

1	ATGGAATTTT	TCATTATCTT	GCTGGCAGCC	GTCGTTGTTT	TCGGCTTCAA
51	ATCCTTTGTT	GTCAATCCAC	AGCAGGAAGT	CCACGTTGTC	GAAAGGCTCG
101	GGCGTTTCCA	TCGCGCCCTG	ACGGCCGGTT	TGAATATTTT	GATTCCCTTT
151	ATCGACCGCG	TCGCCTACCG	CCATTCGCTG	AAAGAAATCC	CTTTAGACGT
201	ACCCAGCCAG	GTCTGCATCA	CGCGCGACAA	TACGCAGCTG	ACTGTTGACG
251	GTATCATCTA	TTTCCAAGTA	ACCGACCCCA	AACTCGCCTC	ATACGGTTTCG
301	AGCAACTACA	TTATGGCGAT	TACCCAGCTT	GCCCAAACGA	CGCTGCGTTC
351	CGTTATCGGG	CGTATGGAAT	TGGACAAAAC	GTTTGAAGAA	CGCGACGAAA
401	TCAACAGCAC	CGTCGTCTCC	GCCCTCGATG	AAGCCGCCGG	AGCTTGGGGT
451	GTGAAGGTTT	TGCGTTATGA	GATTAAAGAC	TTGGTTCCGC	CGCAAGAAAT
501	CCTTCGCTCA	ATGCAGGCGC	AAATTACTGC	TGAACGCGAA	AAACGCGCCC
551	GTATCGCCGA	ATCCGAAGGT	CGTAAAATCG	AACAAATCAA	CCTTGCCAGT
601	GGTCAGCGCG	AAGCCGAAAT	CCAACAATCC	GAAGGCGAGG	CTCAGGCTGC
651	GGTCAATGCG	TCAAATGCCG	AGAAAATCGC	CCGCATCAAC	CGCGCCAAAG
701	GTGAAGCGGA	ATCCTTGCGC	CTTGTGCGG	AAGCCAATGC	CGAAGCCATC
751	CGTCAAATTG	CCGCCGCCCT	TCAAACCCAA	GGCGGTGCGG	ATGCGGTCAA
801	TCTGAAGATT	GCGGAACAAT	ACGTCGCCGC	GTTCAACAAT	CTTGCCAAAG
851	AAAGCAATAC	GCTGATTATG	CCCGCAATG	TTGCCGACAT	CGGCAGCCTG
901	ATTTCTGCCG	GTATGAAAAT	TATCGACAGC	AGCAAACCG	CCAAATAA

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This corresponds to the amino acid sequence <SEQ ID 19; ORF 519-1.a>:

a519-1.pep.

```

1  MEFFFIILLAA VVVFGEKSFV VIPQQEVHV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHS LKEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEEAQAANA SNAEKIARIN RAKGEAESLR LVAEANAIAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*
```

m519-1/a519-1 ORFs 519-1 and 519-1.a showed a 99.0% identity in 315 aa overlap

	10	20	30	40	50	60
a519-1.pep	MEFFFIILLAAVVVFGEKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHS					
m519-1	MEFFFIILLVAVAVFGEKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHS					
	10	20	30	40	50	60
	70	80	90	100	110	120
a519-1.pep	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
m519-1	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
	70	80	90	100	110	120
	130	140	150	160	170	180
a519-1.pep	RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE					
m519-1	RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE					
	130	140	150	160	170	180
	190	200	210	220	230	240
a519-1.pep	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAANASNAEKIARINRAKGEAESLR					
m519-1	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAANASNAEKIARINRAKGEAESLR					
	190	200	210	220	230	240
	250	260	270	280	290	300
a519-1.pep	LVAEANAIAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
m519-1	LVAEANAIAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
	250	260	270	280	290	300
	310					
a519-1.pep	ISAGMKIIDSSKTAKX					
m519-1	ISAGMKIIDSSKTAKX					
	310					

576 and 576-1 gnm22.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 20>:

m576.seq.. (partial)

```

1  ..ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
51  GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
101 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAAATGAC CGAAGAGCAG
151 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAAGCCGT
201 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
```

m576.pgp.. (partial)

g576.seq.. (partial)

q576.pep..(partial)

Homology with a predicted ORF from *N. gonorrhoeae*

	10	20	30	40	50	60
m576.pep	MQQASYAMGVDIGRSLKQMKEQGAIEDLKVFTEAMQAVYDGKEIKMTEEQAQEVMMKFLQ					
g576	:					
	10	20	30	40	50	
	MGVDIGRSLKQMKEQGAIEDLKVFTEAMQAVYDGKEIKMTEEQAQEVMMKFLQ					
	70	80	90	100	110	120
m576.pep	EQQAKAVEKHKADAKANKEKGEAFLKENAAKDGVKTTASGLQYKITKQEGEKQPTKDDIV					
g576	:					
	EQQAKAVEKHKADAKANKEKGEAFLKENAAEDGVKTTASGLQYKITKQEGEKQPTKDDIV					
	60	70	80	90	100	110

```
a576.seq
1  ATGAACACCA TTTTCAAAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
51  ACTTTCCGCG TGCGGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAA
101 CTGCCCGCGC TCTTCCGCG CAGGGCGACA CCTCTTCGAT CGGCACGACG
151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
201 GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
251 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAAATGAC CGAAGAGCAG
301 GCTCAGGAAG TCATGTATGAA ATTCTTCGAG GAACAACAGG CTAAGGCCGT
351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GCGGAAGCCT
401 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
451 CTGCAATACA AAATACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
551 TCGACAGCAG CAAGGCCAAC GCGGCCCGCG TCACTTCCC TTTGAGCCAA
601 GTGATTCTGG GTTGGACCGA AGGCGTACAG TCTCTGAAAG AAGGCGCGCA
651 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCGG
701 GCGACAAAAT CGGCCCGAAC GCCACTTTGG TATTTGATGT GAAACTGGTC
751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAAGTCGA
801 CATCAAAAAA GTAAATTAA
```

a576.pep

1	MNTIFKISAL	TLSAALALSA	CGKKEAAPAS	ASEPAAASSA	QGDTSSIGST
51	MQQASYAMGV	DIGRSLQOMK	EQGAEIDLKV	FTEAMQAVYD	GKEIKMTEEQ
101	AQEVMMKFLQ	EQQAKAVEKH	KADAKANKEK	GEAFLKENAA	KDGVKTTASG
151	LQYKITKQGE	GKQPTKDDIV	TVEYEGRLID	GTVFDSSKAN	GGPVTFLPSQ
201	VILGWTEGVQ	LLKEGGEATF	YIPSNLAYRE	QGAGDKIGPN	ATLVFDVKLV
251	KIGAPENAPA	KOPAADOVDD	VN*		

[illegible]

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	160	170	180	190	200	210
m576.pep	VIPGWTEGVQLKEGGEATFYIPSNLAYREQGAGDKIGPNATLVFDVKLVKIGAPENAPA					
a576	VILGWTEGVQLKEGGEATFYIPSNLAYREQGAGDKIGPNATLVFDVKLVKIGAPENAPA					
	210	220	230	240	250	260
	220					
m576.pep	KQPAQVDIKKVN					
a576	KQPAQVDIKKVN					
	270					

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 26>:

```
m576-1.seq
1  ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
51  ACTTTCCGCC TGCGGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAAC
101 CTGCCGCCGC TTCTCCCGCG CAGGGCGACA CCTCTTCGAT CGGCAGCACG
151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
201 GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
251 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
301 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAAGCCGT
351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
401 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
451 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
551 TCGACAGCAG CAAAGCCAAC GGCGGCCCGG TCACCTTCCC TTTGAGCCAA
601 GTGATTCGGG GTTGGACCGA AGGCGTACAG CTTCTGAAAG AAGGCGGCGA
651 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCGG
701 GCGACAAAAT CGGTCCGAAC GCCACTTTGG TATTTGATGT GAAACTGGTC
751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAAGTCGA
801 CATCAAAAAA GTAAATTAA
```

This corresponds to the amino acid sequence <SEQ ID 27; ORF 576-1>:

```
m576-1.pep
1  MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAASSA QGDTSSIGST
51  MQQASYAMGV DIGRSLQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
101 AQEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
151 LQYKITQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
201 VIPGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFDVKLV
251 KIGAPENAPA KQPAQVDIKK VN*
```

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 28>:

```
g576-1.seq
1  ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
51  ACTTTCCGCC TGCGGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAAC
101 CTGCCGCCGC TTCTGCCGCG CAGGGCGACA CCTCTTCAAT CGGCAGCACG
151 ATGCAGCAGG CAAGCTATGC AATGGGCGTG GACATCGGAC GCTCCCTGAA
201 ACAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGATG
251 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
301 GCCCAGGAAG TGATGATGAA ATTCCTGCAG GAGCAGCAGG CTAAAGCCGT
351 AGAAAAACAC AAGGCGGATG CGAAGGCCAA CAAAGAAAAA GGCGAAGCCT
401 TCCTGAAGGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGT
451 CTGCAGTACA AAATCACCAA ACAGGGTGAA GGCAAACAGC CGACCAAAGA
501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACCGTAT
551 TCGACAGCAG CAAAGCCAAC GGCGGCCCGG CCACCTTCCC TTTGAGCCAA
601 GTGATTCGGG GTTGGACCGA AGGCGTACGG CTTCTGAAAG AAGGCGGCGA
651 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCGG
701 GCGAAAAAAT CGGTCCGAAC GCCACTTTGG TATTTGACGT GAAACTGGTC
751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG ATCAAGTCGA
```

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801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 29; ORF 576-1.ng>:

g576-1.pep
 1 MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAASAA QGDTSSIGST
 51 MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTDAMQAVYD GKEIKMTEEQ
 101 AQEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
 151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPATFPLSQ
 201 VIPGWTEGVR LLKEGGEATF YIPSNLAYRE QGAGEKIGPN ATLVFDVKLV
 251 KIGAPENAPA KQPDQVDIKK VN*

g576-1/m576-1 ORFs 576-1 and 576-1.ng showed a 97.8% identity in 272 aa overlap

	10	20	30	40	50	60
g576-1.pep	MNTIFKISAL	TLSAALALSA	CGKKEAAPAS	ASEPAASAA	QGDTSSIGST	MQQASYAMGV
m576-1	MNTIFKISAL	TLSAALALSA	CGKKEAAPAS	ASEPAASSA	QGDTSSIGST	MQQASYAMGV
	10	20	30	40	50	60
g576-1.pep	70	80	90	100	110	120
	DIGRSLKQMK	EQGAEIDLKV	FTDAMQAVYD	GKEIKMTEEQ	AQEVMMKFLQ	EQQAKAVEKH
m576-1	DIGRSLKQMK	EQGAEIDLKV	FTDAMQAVYD	GKEIKMTEEQ	AQEVMMKFLQ	EQQAKAVEKH
	70	80	90	100	110	120
g576-1.pep	130	140	150	160	170	180
	KADAKANKEK	GEAFLENAA	KDGVKTTASG	LQYKITKQGE	GKQPTKDDIV	TVEYEGRLID
m576-1	KADAKANKEK	GEAFLENAA	KDGVKTTASG	LQYKITKQGE	GKQPTKDDIV	TVEYEGRLID
	130	140	150	160	170	180
g576-1.pep	190	200	210	220	230	240
	GTVPDSSKAN	GGPATFPLSQ	VIPGWTEGVR	LLKEGGEATF	YIPSNLAYRE	QGAGEKIGPN
m576-1	GTVPDSSKAN	GGPATFPLSQ	VIPGWTEGVR	LLKEGGEATF	YIPSNLAYRE	QGAGEKIGPN
	190	200	210	220	230	240
g576-1.pep	250	260	270			
	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
m576-1	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
	250	260	270			

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 30>:

a576-1.seq
 1 ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
 51 ACTTTCCGCC TGCGGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAAC
 101 CTGCCGCCGC TTCTTCCGCG CAGGGCGACA CCTCTTCGAT CGGCAGCACG
 151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
 201 GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
 251 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
 301 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAAGCCGT
 351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
 401 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
 451 CTGCAATACA AAATACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
 501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
 551 TCGACAGCAG CAAAGCCAAC GCGCGCCCGG TCACCTTCCC TTTGAGCCAA
 601 GTGATTCTGG GTTGGACCGA AGGCGTACAG CTTCTGAAAG AAGGCGGCGA
 651 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCAG
 701 GCGACAAAAT CGGCCCGAAC GCCACTTGG TATTGTATGT GAAACTGGTC

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751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAAGTCGA
 801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 31; ORF 576-1.a>:

a576-1.pep
 1 MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAASSA QGDTSSIGST
 51 MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
 101 AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
 151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
 201 VILGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFDVKLV
 251 KIGAPENAPA KQPAQVDIKK VN*

a576-1/m576-1 ORFs 576-1 and 576-1.a 99.6% identity in 272 aa overlap

	10	20	30	40	50	60
a576-1.pep	MNTIFKISAL	TLSAALALS	SACGKKEA	PASASEPA	ASSAQGDT	SSIGSTMQ
m576-1	MNTIFKISAL	TLSAALALS	SACGKKEA	PASASEPA	ASSAQGDT	SSIGSTMQ
	10	20	30	40	50	60
	70	80	90	100	110	120
a576-1.pep	DIGRSLKQ	MKEQGAEI	DLKVFTEA	MQAVYDG	KKEIKMT	EEQAQEV
m576-1	DIGRSLKQ	MKEQGAEI	DLKVFTEA	MQAVYDG	KKEIKMT	EEQAQEV
	70	80	90	100	110	120
	130	140	150	160	170	180
a576-1.pep	KADAKANKE	GEAFLENA	AKDGVKTT	ASGLQYKI	TQGEKGQ	PTKDDIV
m576-1	KADAKANKE	GEAFLENA	AKDGVKTT	ASGLQYKI	TQGEKGQ	PTKDDIV
	130	140	150	160	170	180
	190	200	210	220	230	240
a576-1.pep	GTVFDSSKA	NGGPVTF	PLSQVILG	WTEGVQL	LKEGGEAT	FYIPSNL
m576-1	GTVFDSSKA	NGGPVTF	PLSQVILG	WTEGVQL	LKEGGEAT	FYIPSNL
	190	200	210	220	230	240
	250	260	270			
a576-1.pep	ATLVFDVK	LVKIGAP	ENAPAKQ	PAQVDIK	KVNVN	
m576-1	ATLVFDVK	LVKIGAP	ENAPAKQ	PAQVDIK	KVNVN	
	250	260	270			

919 and 919-2 gnm43.seq

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 32>:

m919.seq
 1 ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TACGGCATCG CCGCCGCCAT
 51 CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
 101 CATCCGTCAT CAACGGCCCG GACCGGCCCG TCGGCATCCC CGACCCCGCC
 151 GGAACGACGG TCGGCGCGCG CGGGGCCGTC TATACCGTTG TACCGCACCT
 201 GTCCCTGCCC CACTGGGCGG CGCAGGATTT CGCCAAAAGC CTGCAATCCT
 251 TCCGCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
 301 TGCGCCCAAG CCTTTCAAAC CCCCCTCCAT TCCTTTCAGG CAAAACAGTT
 351 TTTTGAACGC TATTTACGCG CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG

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401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTTCG GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACCGCCG ACCTCTCCcG ATTCCCcATC ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCgATACT CGGTTACGCC
751 GAAGACCCcT TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCyTA CGTTTCCATC GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAACCTCGAC AAACCTCCAT GCAGGGCATT AAGTCTTATA TGCGGCAAAA
951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCcAGC TATATCTTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCCTGTCCG CGCACTGGGC
1051 ACGCGCTGA TGGGGGAATA TGCCGCGCGA GTCGACCGGC ACTACATTAC
1101 CTTGGGTGCG CCCTTATTTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCGAACT
1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTC CTACCCAACG
1301 GTATGAAGCC CGAATACCGc CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 33; ORF 919>:

m919.pep

```

1 MKKYLFRAL YGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51 GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
151 RTAQRFPYIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQGSRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELAGS SNDGPVAGLG
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

```

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 34>:

m919-2.seq

```

1 ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TACGGCATCG CCGCCGCCAT
51 CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCCG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGGCGGCGG CGGGGCCGTC TATACCGTTG TACCGCACCT
201 GTCCTTGCCC CACTGGGCGG CGCAGGATTT CGCCAAAAGC CTGCAATCCT
251 TCCGCCTCGG CTGCGCCAAT TTGAAAACC GCCAAGGCTG GCAGGATGTG
301 TCGGCCAAG CCTTTCAAAC CCCCGTCCAT TCCTTTCAGG CAAAACAGTT
351 TTTTGAACGC TATTTACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTTCG GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACCGCCG ACCTCTCCCG ATTCCCcATC ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCgATACT CGGTTACGCC
751 GAAGACCCcT TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCCTA CGTTTCCATC GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAACCTCGAC AAACCTCCAT GCAGGGCATT AAGTCTTATA TGCGGCAAAA
951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCcAGC TATATCTTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCCTGTCCG CGCACTGGGC
1051 ACGCGCTGA TGGGGGAATA TGCCGCGCGA GTCGACCGGC ACTACATTAC
1101 CTTGGGTGCG CCCTTATTTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC

```


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1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCGAACT
 1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTC CTACCCAACG
 1301 GTATGAAGCC CGAATACCGC CCGTAA

This corresponds to the amino acid sequence <SEQ ID 35; ORF 919-2>:

m919-2.pep

1 MKKYLFRAL YGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
 51 GTTVGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
 101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDGR
 151 RTAQARFPIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
 201 HTADLSRFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
 251 EDPVELFFMH IQSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
 301 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELAGS SNDGPVGALG
 351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 36>:

g919.seq

1 ATGAAAAAAC ACCTGCTCCG CTCCGCCCTG TACGGcatCG CCGCCgccaT
 51 CctcgCCGCC TGCCAAAgca gGAGCATCCA AACCTTTCCG CAACCCGACA
 101 CATCCGTCAT CAACGGCCCG GACCGGCCGG CCGGCATCCC CGACCCCGCC
 151 GGAACGACGG TTGCCGCGCG CGGGGCCGTC TATACCGTTG TGCCGCACTT
 201 GTCCATGCCC CACTGGGCGG CGCaggATTT TGCCAAAAGC CTGCAATCCT
 251 TCCGCTCCG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
 301 TGCGCCAAG CCTTCAAAC CCCCCTGCAT TCCTTTCAGG CAAAGcGgTT
 351 TTTTGAACGC TATTTACGC cgtGGCagg tgcaggcaAC GGAAGcCTTG
 401 Caggtaacgt TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGCGAGG
 451 CGGACGGAAC GGGCCCGCTT CCCGATTAC GGTATTCCCG ACGATTTTAT
 501 CTCCGTCCCG CTGCTGCGG GTTTGCGGGG CGGAAAAAAC CTTGTCCGCA
 551 TCAGGCAGac ggGGAAAAAC AGCGGCACGA TCGACAATGC CGGCGGCACG
 601 CATAACGCGG ACCTCTCCCG ATTCCCCATC ACCGCGCGCA CAACGGcaat
 651 caaaGCAGG TTTGAaggAA GCCGCTTCCT CCCTTACCAC ACGCGCAACC
 701 AAAtcaacGG CGGCgcgcTT GACGGCAAag cccCATCCT CggttacgcC
 751 GAagaccCcG tccgaacttTT TTTCATGCAC AtccaaggCT CGGGCCGCTT
 801 GAAAAACCCg tccggcaaat acatCCGCA cggatagcc gacAAAAACG
 851 AACAtccgTa tgtttccatc ggACGctata TGGCGGACAA AGGCTACCTC
 901 AAGctcgggc agACCTCGAT GCAGGgcac aaagcCTATA TGCGGCAAAA
 951 TCCGCAACGC CTCGCGAAG TTTTGGGTCA AAACCCGAGC TATATCTTTT
 1001 TCCGCGAGCT TGCCGGAAGC GGCAATGAGG GCCCGGTCGG CGCACTGGGC
 1051 ACGCCACTGA TGGGGGAATA CGCCGGCGCA ATCGACCGGC ACTACATTAC
 1101 CTTGGGCGCG CCCTTATTTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
 1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CAGGCAGCGC GATCAAAGGC
 1201 GCGGTGCGCG TGGATTATTT TTGGGGTTAC GGCGACGAAG CCGGCGAACT
 1251 TGCCGGCAAA CAGAAAACCA CGGGATACGT CTGGCAGCTC CTGCCCAACG
 1301 GCATGAAGCC CGAATACCGC CCGTGA

This corresponds to the amino acid sequence <SEQ ID 37; ORF 919.ng>:

g919.pep

1 MKKHLLRSAL YGIAAAILAA CQSRSIQTFP QPDTSVINGP DRPAGIPDPA
 51 GTTVAGGAV YTVVPHLSMP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
 101 CAQAFQTPVH SFQAKRFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDGR
 151 RTERARFPIY GIPDDFISVP LPAGLRGGKN LVRIRQTGKN SGTIDNAGGT
 201 HTADLSRFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
 251 EDPVELFFMH IQSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
 301 KLGQTSMQGI KAYMRNPQR LAEVLGQNPS YIFFRELAGS GNEGPVGALG
 351 TPLMGEYAGA IDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

		10	20	30	40	50	60
m919.pep	MKKYLFRAALYGIAAAILAACQSKSIQTFFQPDTSVINGPDRPVGIPDPAGTTVGGGGAV ::: : : : : : : : : : : : : : : : : :						
g919	MKKHLLRSALYGIAAAILAACQSRSIQTFPPQDTSVINGPDRPAGIPDPAGTTVAGGGAV 10 20 30 40 50 60						
		70	80	90	100	110	120
m919.pep	YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWDVCAQAFQTPVHSFQAQKOFFER : : : : : : : : :						
g919	YTVVPHLSMPHWAAQDFAKSLQSFRLGCANLKNRQGWDVCAQAFQTPVHSFQAKRFFER 70 80 90 100 110 120						
		130	140	150	160	170	180
m919.pep	YFTPWQVAGNGSLAGTGTGYEYPVLKGDGRRTAQARFPITYGIPDDFISVPLPAGLRSGKA : : : : : : : : :						
g919	YFTPWQVAGNGSLAGTGTGYEYPVLKGDGRRTERARFPITYGIPDDFISVPLPAGLRGGKN 130 140 150 160 170 180						
		190	200	210	220	230	240
m919.pep	LVRIRQTGKNSGTIDNTGGTHTADLSRFPI TARTTAIKGRFEFSRFLPYHTRNQINGGAL : : : : : : : : :						
g919	LVRIRQTGKNSGTIDNAGGTHTADLSRFPI TARTTAIKGRFEFSRFLPYHTRNQINGGAL 190 200 210 220 230 240						
		250	260	270	280	290	300
m919.pep	DGKAPILGYAEDPVLEFFMHIQSGSRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL : : : : : : : : :						
g919	DGKAPILGYAEDPVLEFFMHIQSGSRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL 250 260 270 280 290 300						
		310	320	330	340	350	360
m919.pep	KLGQTSMQGIKS YMRQN PQRLAEVLGQNPSYIFFREL AGSSNDGPVGALGTPLMGEYAGA : : : : : : : : :						
g919	KLGQTSMQGIKAYMRQN PQRLAEVLGQNPSYIFFREL AGSGNEGVPVGALGTPLMGEYAGA 310 320 330 340 350 360						
		370	380	390	400	410	420
m919.pep	VDRHYITLGAPL FVATAHPVTRKALNRLIM AQDTGS A IKGAVRV D YFWGYGDEAGELAGK : : : : : : : : : :						
g919	IDRHYITLGAPL FVATAHPVTRKALNRLIM AQDTGS A IKGAVRV D YFWGYGDEAGELAGK 370 380 390 400 410 420						
		430	440				
m919.pep	QKT TG YV WQLLP NGMKPEYRPX : : : : : : : : :						
g919	QKT TG YV WQLLP NGMKPEYRPX 430 440						

a919.seq

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1  ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TCGGGCATCG CCGCCGCCAT
51  CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGGCGGCGG CGGGGCCGTT TATACCGTTG TGCCGCACCT
201 GTCCCTGCCC CACTGGGCGG CGCAGGATTT CGCCAAAAGC CTGCAATCCT
251 TCCGCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
301 TCGGCCCAAG CCTTTCAAAC CCCCCTCCAT TCCGTTCCAG CAAAACAGTT
351 TTTTGAACGC TATTTCACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAGCCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTTAC GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACGCGCG ACCTCTCCCA ATTCCCCTATC ACTGCGCGCA CAACGGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCATACT CCGTTACGCC
751 GAAGACCCCG TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCCTA CGTTTCCATC GGACGCTATA TGGCGGACAA AGGCTACCTC
901 AAGCTCGGGC AGACCTCGAT GCAGGGCATC AAAGCCTATA TGCAGCAAAA
951 CCCGCAACGC CTCGCCAAG TTTTGGGGCA AAACCCAGC TATATCTTTT
1001 TCCGAGAGCT TACCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGCGAGTA CGCCGGCGCA GTCGACCGGC ACTACATTAC
1101 CTTGGGCGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCGAACT
1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTT CTGCCCAACG
1301 GTATGAAGCC CGAATACCGC CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 39; ORF 919.a>:

```

a919.pep
1  MKKYLFRAL CGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51  GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SVQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDE
151 RTAQAQRFPI YIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
201 HTADLSQFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQGI KAYMQNPQR LAEVLGQNPS YIFFRELTGS SNDGPVGALG
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

```

m919/a919 ORFs 919 and 919.a showed a 98.6% identity in 441 aa overlap

```

m919.pep      10      20      30      40      50      60
MKKYLFRALYGI AAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTVGGGGAV
|||||
a919          10      20      30      40      50      60
MKKYLFRALCGI AAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTVGGGGAV

m919.pep      70      80      90      100     110     120
YTVVPHLSLPHW AAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
|||||
a919          70      80      90      100     110     120
YTVVPHLSLPHW AAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSVQAKQFFER

m919.pep     130     140     150     160     170     180
YFTPWQVAGNGL AGTVTGYEYEPVLKGDDERTAQAQRFPIYIPDDFISVPLPAGLRSGKA
|||||
a919         130     140     150     160     170     180
YFTPWQVAGNGL AGTVTGYEYEPVLKGDDERTAQAQRFPIYIPDDFISVPLPAGLRSGKA

m919.pep     190     200     210     220     230     240
LVRIRQTGKNSGT IDNTGGTHTADLSRFPITARTTAIKGRFEGRSFLPYHTRNQINGGAL
|||||
a919         190     200     210     220     230     240
LVRIRQTGKNSGT IDNTGGTHTADLSRFPITARTTAIKGRFEGRSFLPYHTRNQINGGAL
|||||

```

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```

a919      LVRI RQTG KNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
           190      200      210      220      230      240

           250      260      270      280      290      300
m919.pep  DGKAPILGYAEDPVELFFMHQSGRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL
           |||||||
a919      DGKAPILGYAEDPVELFFMHQSGRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL
           250      260      270      280      290      300

           310      320      330      340      350      360
m919.pep  KLGQTSMQGIKSYMRQNPQRLAEVLGQNPSYIFFRELAGSSNDGPGVGLGTPLMGEYAGA
           |||||||:|:|||||
a919      KLGQTSMQGIKAYMQQNPQRLAEVLGQNPSYIFFREL TGSNDGPGVGLGTPLMGEYAGA
           310      320      330      340      350      360

           370      380      390      400      410      420
m919.pep  VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELAGK
           |||||||
a919      VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELAGK
           370      380      390      400      410      420

           430      440
m919.pep  QKTTGYVWQLLPNGMKPEYRPX
           |||||||
a919      QKTTGYVWQLLPNGMKPEYRPX
           430      440

```

121 and 121-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 40>:

m121.seq

```

1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCAGCG CTTTACCCCC TACCCCGGCA GGTTACGCCG CCAATTGCTG
151 GATTTCAGAG ACACAGGCGC AGACGAACTG CACCGCAGCA GGATTTTGTC
201 GCAAGAACTC AGCCGCCTAT ATGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCACCCTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCGAC  ACGCGCCGGA ACACGGTTAC AGCATACAGC TTGCCGATT
351 GCCGCTGCTG GCGxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
401 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
451 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
501 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
551 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
601 xxxxxxCAGC TTCCTTACGA CAAAACGGT  GCAAAGTCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCCACCCG TATTTGCGAC
701 AACGCCACCC TAAAGCACG  GGGCGCGAAC TGTTTGCCAT AAATTGGCTC
751 GAAACCTACC TTGACGCGCG CGAAAACCGA TACGACGTAT TGCGGACGCT
801 TTCCCGTTTT ACCGCGCAAA CCGTTTGCGA CGCCGTCTCA CACGCAGCGG
851 CAGATGCCCG TCAATGTAC  ATTTGCGACG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGCGAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGnATTG
1001 CGTGGTTGGC GCGGTGTTGG ATTAATCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG AnCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 41; ORF 121>:

m121.pep

```

1  METQLYIGIM SGTSMDGADA VLIRMDGGKW LGAEGHAFTP YPGLRRQLL
51  DLQDTGADEL HRSRILSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ

```

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```

101 TVRHAPHEGY SIQLADLPLL Axxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
151 xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
201 xxQLPYDKNG AKSAQGNILP QLLDRLLAHP YFAQRHPKST GRELFAINWL
251 ETYLDGGENR YDVLRTLRSR TAQTVCDAYS HAAADARQMY ICDGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 42>:

g121.seq

```

1 ATGGAAACAC AGCTTTACAT CGGCATTATG TCGGGAACCA GTATGGACGG
51 GGCGGATGCC GTGCTGGTAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCAGCG CTTTACCCCG TACCCTGACC GGTTGCGCCG CAAATTGCTG
151 GATTTCGAGG ACACAGGCAC AGACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCCTGT ACGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCTCCGTGC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCCGAC ACGCGCCGGA ACACGGTtac AGCATACAGC TTGCCGATTT
351 GCCGTGCTG GCGGAACTGa cgcggatttT TACCGTCggc gacttcCGCA
401 GCCGCGACCT TGCTGCCGGC GGacaAGGTG CGCCGCTCGT CCCCCTCTTT
451 CACGAAGCCC TGTTCGCGA TGACAGGGAA ACACGCGTGG TACTGAACAT
501 CGCGGGGATT GCCAACATCA GCGTACTCCC CCCCAGCGCA CCGCCTTCG
551 GCTTCGACAC AGGGCCGGGC AATATGCTGA TGGAcgcgtg gacgcaggca
601 cacTGGcagc TGCTTACGA CAAAaAcggt gcAAAGGcgg cacAAGGCAA
651 catatTGCcg cAACTGCTCG gcaggctGCT CGCCcaccCG TATTTCTCAC
701 AACCCcacc aaAAAGCAG GGgcGCGaac TgtttgcccT AAattggctc
751 gaaacctAcc ttgacgcgcg cgaaaaccga tacgacgtat tgcggacgct
801 ttcccgattc accgcgcaaA ccgTttggga cgcggtetca CACGCAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACGCGCGAA CTGAACCTCG ATCCTCAATG GGTGGAGGCG gccgCATttg
1001 cgtggttggC GCGGTGTTGG ATTAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCGACCGGCG CATCCAAACC GTGTATTCTG GGCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 43; ORF 121.ng>:

g121.pep

```

1 METQLYIGIM SGTSMGADA VLVRMDGKW LGAEGHAFTP YPDLRRLKLL
51 DLQDTGTDEL HSRMLSQEL SRLYAQTAAE LLCSQNLAPC DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLL AELTRIFTVG DFRSRDLAAG GQGAPLVPF
151 HEALFRDDRE TRVVLNIGGI ANISVLPFGA PAFGFDTPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLGRLLAHP YFSQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLRSR TAQTVWDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AAFWLAACW INRIPGSPHK
351 ATGASKPCIL GAGYYY*

```

ORF 121 shows 73.5% identity over a 366 aa overlap with a predicted ORF (ORF121.ng) from *N. gonorrhoeae*:

m121/g121

	10	20	30	40	50	60
m121.pep	METQLYIGIMSGTSMGADAVLIRMDGKKWLGAEGHAFTPYPGRRLRQLLDLQDTGADEL					
	: : : : :					
g121	METQLYIGIMSGTSMGADAVLVRMDGKKWLGAEGHAFTPYPDLRRLKLLDLQDTGTDEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121.pep	HSRRLSQELSRLYAQTAAELLCSQNLAPSDITALGCHGQTVRHAPHEGYSIQLADLPLL					
	: : : : :					
g121	HSRRLSQELSRLYAQTAAELLCSQNLAPCDITALGCHGQTVRHAPHEGYSIQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180

```

a121.seq
1  ATGGAACAC  AGCTTTACAT  CGGCATCATG  TCGGGAACCA  GCATGGACGG
51  GGCGGATGCC  GTACTGATAC  GGATGGACGG  CGGCAAATGG  CTGGGCGCGG
101 AAGGCGACGC  CTTTACCCCC  TACCCCGGCA  GCTTACGCGC  GAAATTGCTG
151 GATTTGCAGG  ACACAGGCGC  GGACGAAC TG  CACCGACGCA  CATGTTTGTC
201 GCAAGAACTC  AGCCGCCTGT  ACGCGCAAAC  CGCCGCCGAA  CTGCTGTGCA
251 GTCAAACCT  CGCGCCGTCC  GACATTACCG  CCCTCGGCTG  CCACGGGCAA
301 ACCGTCAGAC  ACGCGCCGGA  ACACAGTTAC  AGCGTACAGC  TTGCCGATTT
351 GCCGTGCTG  GCGGAACGGA  CTCAGATTTT  TACCGTCGGC  GACTTCCGCA
401 GCCGCGACCT  TGC GGCCGGC  GGACAAGGCG  CGCGCTCGT  CCCC GCCTTT
451 CACGAAGCCC  TGTTCCGCGA  CGACAGGGAA  ACACGCGCGG  TACTGAACAT
501 CGGCGGGATT  GCCAACATCA  GCGTACTCCC  CCCC GACGCA  CCCG CCTTCG
551 GCTTCGACAC  AGGACCGGGC  AATATGCTGA  TGGACGCGTG  GATGCAGGCA
601 CACTGGCAGC  TTCTTTACGA  CAAAACCGGT  CGAAGGCGG  CACAAGGCAA
651 CATATTGCCG  CAACTGCTCG  ACAGGCTGCT  CGCCACCCG  TATTTGCGAC
701 AACCCACCC  TAAAAGCACG  GGGCGCGAAC  TGTTTGCCCT  AAATTGGCTC
751 GAAACCTACC  TTGACGGCGG  CGAAAACCGA  TACGACGTAT  TCGG GACGCT
801 TTCCCGATT  ACCGCGCAA  CCGTTTTCGA  CGCCGTCTCA  CACGACGCGT
851 CAGATGCCCG  TCAAAATGTAC  ATTTGCGGCG  CGGGCATCCG  CAATCCTGTT
901 TTAATGGCGG  ATTTGGCAGA  ATGTTTCGGC  ACACGCGTTT  CCCTGCACAG
951 CACCGCCGAA  CTGAACCTCG  ATCCGCAATG  GGTAGAAGCC  GCCGCGTTTC
1001 CATGATGGC  GCGGTGTTGG  GTCAACCGCA  TTCCCGGTAG  TCCGCACAAA
1051 GCAACCGGCG  CATCAAACC  GTGTATTCTG  GGCGCGGGAT  ATTATTATTG
1101 A

```

```

a121.pep
  1  METQLYIGIM  SGTSMDGADA  VLIRMDGGKW  LGAEGHAFTP  YPGRLLRRKLL
  51  DLQDTGADEL  HRSRMLSQEL  SRLYAQTAAE  LLCSQNLAFT  DITALGCHGQ
101  TVRHAPESHSY  SVQLADPLLL  AERTQIFTVG  DFRSRDLAAG  QGAPLVPFAF
151  HEALFRDDRE  TRAVLNIGGI  ANISVLPPDA  PAFGFDTPGP  NMLMDAWMQA
201  HWQLPYDKNG  AKAAQGNILP  QLLDRLLAHP  YFAQPHPKST  GRELFALNWL
251  ETYLDGGENR  YDVLRLTSRF  TAQTVFDAVS  AAADARQOMY  ICGGGIRNPV
301  LMADLAECFG  TRVSLHSTAE  LNLDPQWVEA  AAFAWMAACW  VNRIPGSPHK

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351 ATGASKPCIL GAGYYY*

m121/a121 ORFs 121 and 121.a 74.0% identity in 366 aa overlap

m121.pep	10	20	30	40	50	60
	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTPYPGRLRRQLLDLQDTGADEL					
a121	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTPYPGRLRRKLLDLQDTGADEL					
	10	20	30	40	50	60
m121.pep	70	80	90	100	110	120
	HRSRILSQELSRLYAQTAELLCSQNLA PSDITALGCHGQTVRHAPENHGYSIQLADLPLL					
a121	HRSRILSQELSRLYAQTAELLCSQNLA PSDITALGCHGQTVRHAPENHGYSVQLADLPLL					
	70	80	90	100	110	120
m121.pep	130	140	150	160	170	180
	XX					
a121	AERTQIFTVGDFRSRDLAAGGQGAPLVPAPFHEALFRDDRETRAVLNIGGIANISVLPPDA					
	130	140	150	160	170	180
m121.pep	190	200	210	220	230	240
	XXXXXXXXXXXXXXXXXXXXXXXXXQLPYDKNGAKSAQGNILPQLLDRLLAHPYFAQRHPKST					
a121	PAFGFDTGPGNMLMDAWMQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	190	200	210	220	230	240
m121.pep	250	260	270	280	290	300
	GRELFAINWLETYLDGGENRYDVLRLTSRFTAQTVCDVSHAAADARQMYICDGGIRNPV					
a121	GRELFAINWLETYLDGGENRYDVLRLTSRFTAQTVFDVSHAAADARQMYICGGGIRNPV					
	250	260	270	280	290	300
m121.pep	310	320	330	340	350	360
	LMADLAECFGTRVSLHSTADLNLDPOWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
a121	LMADLAECFGTRVSLHSTAE LNLDPOWVEAAAFAWMAACWVNRI PGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121.pep	XAGYYYYX					
a121	GAGYYYYX					

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 46>:

m121-1.seq

1	ATGGAAACAC	AGCTTTACAT	CGGCATCATG	TCGGGAACCA	GCATGGACGG
51	GGCGGATGCC	GTACTGATAC	GGATGGACGG	CGGCAAATGG	CTGGGCGCGG
101	AAGGGCAGCG	CTTTACCCCC	TACCCCGGCA	GGTTACGCCG	CCAATTGCTG
151	GATTTCAGAG	ACACAGGCGC	AGACGAACTG	CACCGCAGCA	GGATTTTGTC
201	GCAAGAACTC	AGCCGCCTAT	ATGCGCAAAC	CGCCGCCGAA	CTGCTGTGCA
251	GTCAAAACCT	CGCACCGTCC	GACATTACCG	CCCTCGGCTG	CCACGGGCAA
301	ACCGTCCGAC	ACGCGCCGGA	ACACGGTTAC	AGCATACAGC	TTGCCGATTT
351	GCCGCTGCTG	GCGGAACGGA	CGCGGATTTT	TACCGTCGCG	GA CTTCGCGA
401	GCCGCGACCT	TGCGGCCGGC	GGACAAGGCG	CGCCACTCGT	CCCCGCCTTT
451	CACGAAGCCC	TGTTCCGCGA	CAACAGGGAA	ACACGCGCGG	TACTGAACAT
501	CGGCGGGATT	GCCAACATCA	GCGTACTCCC	CCCCGACGCA	CCCGCCTTCG
551	GCTTCGACAC	AGGGCCGGGC	AATATGCTGA	TGGACGCGTG	GACGCAGGCA
601	CAC TGGCAGC	TTCTTACGA	CAAAAACGGT	GCAAAGGCGG	CACAAGGCAA
651	CATATTGCCG	CAACTGCTCG	ACAGGCTGCT	CGCCCACCCG	TATTTGCGAC
701	AACCCACCCC	TAAAAGCACG	GGGCGCGAAC	TGTTTGCCCT	AAATTGGGCTC
751	GAAACCTACC	TTGACGGCGG	CGAAAACCGA	TACGACGTAT	TGCGGACGCT

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```

801 TTCCCGTTTT ACCGCGCAAA CCGTTTGCGA CGCCGTCTCA CACGCAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGNATTTG
1001 CGTGGTTGGC GCGGTGTTGG ATTAATCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG ANCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 47; ORF 121-1>:

```

m121-1.pep
  1 METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEGHAFTP YPGRLLRRQLL
 51 DLQDTGADEL HRSRILSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLL AERTRIFTVG DFRSRDLAAG GQGAPLVPAF
151 HEALFRDNRE TRAVLNIGGI ANISVLPPDA PAFGFDTPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVCDAYS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

m121-1/g121 ORFs 121-1 and 121-1.ng showed a 95.6% identity in 366 aa overlap

m121-1.pep	10	20	30	40	50	60
	METQLYIGIMSGTSMGDADAVLIRMDGGKW LGAEGHAFTPYPGRLLRRQLLDLQDTGADEL					
g121	METQLYIGIMSGTSMGDADAVLVRMDGGKW LGAEGHAFTPYPDRLLRRKLLDLQDTGTDEL					
	10	20	30	40	50	60
m121-1.pep	70	80	90	100	110	120
	HRSRILSQELSRLYAQTAAELLCSQNLAPSDITALGCHGQTVRHAPHEGYSIQLADLPLL					
g121	HRSRMLSQELSRLYAQTAAELLCSQNLAPCDITALGCHGQTVRHAPHEGYSIQLADLPLL					
	70	80	90	100	110	120
m121-1.pep	130	140	150	160	170	180
	AERTRIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDNRETRAVLNIGGIANISVLPPDA					
g121	AELTRIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDDRETRVVLNIGGIANISVLPPGA					
	130	140	150	160	170	180
m121-1.pep	190	200	210	220	230	240
	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
g121	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLGRLLAHPYFSQPHPKST					
	190	200	210	220	230	240
m121-1.pep	250	260	270	280	290	300
	GRELFALNWLETYLDGGENRYDVLRTLSRF TAQTVCDAYS HAAADARQMYICGGGIRNPV					
g121	GRELFALNWLETYLDGGENRYDVLRTLSRF TAQTVWDAYS HAAADARQMYICGGGIRNPV					
	250	260	270	280	290	300
m121-1.pep	310	320	330	340	350	360
	LMADLAECFGTRVSLHSTADLNLDPQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
g121	LMADLAECFGTRVSLHSTAE LNLDPQWVEA AAXFAWLAACWINRIPGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121-1.pep	XAGYYYYX					
g121	GAGYYYYX					

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The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 48>:

```
a121-1.seq
  1  ATGGAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
 51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101  AAGGGCACGC CTTTACCCCC TACCCCGGCA GGTTACGCCG CAAATTGCTG
151  GATTTCGAGG ACACAGGCGC GGACGAACTG CACCGCAGCA GGATGTTGTC
201  GCAAGAACTC AGCCGCCTGT ACGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251  GTCAAAACCT CGCGCCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301  ACCGTCAGAC ACGCGCCGGA ACACAGTTAC AGCGTACAGC TTGCCGATTT
351  GCCGTGCTG GCGGAACGGA CTCAGATTTT TACCGTCGGC GACTTCCGCA
401  GCCGCGACCT TCGGCGCGGC GGACAAGGCG CGCCGCTCGT CCCCGCCTTT
451  CACGAAGCCC TGTTCGCGA CGACAGGGAA ACACGCGCGG TACTGAACAT
501  CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCAGCGCA CCCGCCTTCG
551  GCTTCGACAC AGGACCGGGC AATATGCTGA TGGACGCGTG GATGCAGGCA
601  CACTGGCAGC TTCCCTTACGA CAAAAACGGT GCAAAGGCGG CACAAGGCAA
651  CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCCACCCG TATTTGCGAC
701  AACCCACCCC TAAAGCAGC GGGCGCGAAC TGTTCGCCCT AAATTGGCTC
751  GAAACCTACC TTGACGCGCG CGAAAACCGA TACGACGTAT TGCGGACGCT
801  TTCCCGATTG ACCGCGCAAA CCGTTTTCGA CGCCGTCTCA CACGCAAGCG
851  CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901  TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951  CACCGCCGAA CTGAACCTCG ATCCGCAATG GGTAGAAGCC GCCGCGTTTCG
1001 CATGGATGGC GCGGTGTTGG GTCAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG GGCGCGGGAT ATTATTATTG
1101 A
```

This corresponds to the amino acid sequence <SEQ ID 49; ORF 121-1.a>:

```
a121-1.pep
  1  METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEGHAFTP YPGRRLRRKLL
 51  DLQDTGADEL HRSRMLSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ
101  TVRHAPHSY SVQLADLPLL AERTQIFTVG DFRSRDLAAG GQGAPLVPF
151  HEALFRDDRE TRAVLNIGGI ANISVLPPDA PAFGFDTGPG NMLMDAWMQA
201  HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251  ETYLDGGENR YDVLRTLSRF TAQTVFDAVS HAAADARQMY ICGGGIRNPV
301  LMADLAECFG TRVSLHSTAE LNLDPQWVEA AFAFWMAACW VNRIPGSPHK
351  ATGASKPCIL GAGYY*
```

m121-1/a121-1 ORFs 121-1 and 121-1.a showed a 96.4% identity in 366 aa overlap

	10	20	30	40	50	60
m121-1.pep	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTYPYGRRLRRQLLDLQDTGADEL					
a121-1	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTYPYGRRLRRKLLDLQDTGADEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121-1.pep	HRSRILSQELSRLYAQTAAELLCSQNLAPSDITALGCHGQTVRHAPHEHGYSIQLADLPLL					
a121-1	HRSRMLSQELSRLYAQTAAELLCSQNLAPSDITALGCHGQTVRHAPHSYSVQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180
m121-1.pep	AERTQIFTVGDFRSRDLAAGGQAPLVPFHEALFRDNRETRAVLNIGGIANISVLPPDA					
a121-1	AERTQIFTVGDFRSRDLAAGGQAPLVPFHEALFRDDRETRAVLNIGGIANISVLPPDA					
	130	140	150	160	170	180
	190	200	210	220	230	240
m121-1.pep	PAFGFDTGPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
a121-1	PAFGFDTGPGNMLMDAWMQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	190	200	210	220	230	240

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	250	260	270	280	290	300
m121-1.pep	GREL FALNWLE TYLDG GENRYD VLR TLSRFTAQ TVCD AVSHAAADARQ MYICGGGIRNPV					
a121-1	GREL FALNWLE TYLDG GENRYD VLR TLSRFTAQ TVFD AVSHAAADARQ MYICGGGIRNPV					
	250	260	270	280	290	300
	310	320	330	340	350	360
m121-1.pep	LMADLAECFGTRVSLHSTADLNLD PQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
a121	LMADLAECFGTRVSLHSTAE LNLD PQWVEAAAFAWMAACWVNRI PGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121-1.pep	XAGYYYYX					
a121	GAGYYYYX					

128 and 128-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 50>:

```

m128.seq (partial)
  1  ATGACTGACA ACGCACTGCT CCATTTGGGC GAAGAACCCC GTTTTGATCA
 51  AATCAAAACC GAAGACATCA AACCCGCCCT GCAAACCGCC ATCGCCGAAG
101  CGCGCGAACA AATCGCCGCC ATCAAAGCCC AAACGCACAC CGGCTGGGCA
151  AACACTGTCTG AACCCCTGAC CGGCATCACC GAACGCGTCG GCAGGATTGT
201  GGGCGTGGTG TCGCACCTCA ACTGCGTCGC CGACACGCCC GAACTGCGCG
251  CCGTCTATAA CGAACTGATG CCCGAAATCA CCGTCTTCTT CACCGAAATC
301  GGACAAGACA TCGAGCTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351  CGAATTCGAC ACCCTCTCCC CCGCACAAAA AACCAACTC AACCAC
  1  TACGCCAGCG AAAAAGTGGC CGAAGCCAAA TACGCGTTCA GCGAAACCGA
 51  wGTCAAAAAA TAyTTCCyG TCGGCAAwGT ATTAACCGGA CTGTTCGCC
101  AAmTCAAAAA ACTmTACGGC ATCGGATTTA CCGAAAAAAC yGTCCCCGTC
151  TGGCACAAG ACGTGCGCTA TtkTGAATTG CAACAAAACG GCGAamCCAT
201  AGCGCGCGTT TATATGGATT TGTACGCACG CGAAGGCAAA CGCGCGCGCG
251  CGTGGATGAA CGACTACAAA GGCCGCCGCC GTTTTTCAGA CGGCACGCTG
301  CAAYTGCCCA CCGCCTACCT CGTCTGCAAC TTCGCCCCAC CCGTCGGCGG
351  CAGGAAGCC CGCyTGAGCC ACGACGAAAT CCTCATCTC TTCCACGAAA
401  CCGGACACGG GCTGCACCAC CTGCTTACCC AAGTGGACGA ACTGGGCGTA
451  TCCGGCATCA ACGGCGTAKA ATGGGACGCG GTCGAACTGC CCAGCCAGTT
501  TATGAAAAAT TTCGTTTGGG AATACAATGT CTTGGCACAA mTGTCAGCCC
551  ACGAAGAAAC CGGcgTTCCC yTGCCGAAAG AACTCTTsGA CAAAwTGCTC
601  GCCGCCAAAA ACTTCCAAsG CGGCATGTTC yTsGTCCGGC AAwTGGAGTT
651  CGCCCTCTTT GATATGATGA TTTACAGCGA AGACGACGAA GGCCGTCTGA
701  AAAACTGGCA ACAGGTTTTA GACAGCGTGC GCAAAAAAGT CGCCGTCATC
751  CAGCCGCCCG AATACAACCG CTTCGCCCTT AGCTTCGGCC ACATCTTCGC
801  AGCGGCTAT TCCGAGCTn ATTACAGCTA CGGTGGGCG GAAGTATTGA
851  GCGCGGACGC ATACGCCGCC TTTGAAGAAA GCGACGATGT CGCCGCCACA
901  GGCAAACGCT TTTGGCAGGA AATCCTCGCC GTCGGGGnAT CGCGCAGCGG
951  nGCAGAAATC TTCAAAGCCT TCCGCGGCCG CGAACCGAGC ATAGACGCAC
1001 TCTTGCGCCA CAGCGGTTTC GACAACGCGG TCTGA

```

This corresponds to the amino acid sequence <SEQ ID 51; ORF 128>:

```

m128.pep (partial)
  1  MTDNALLHLG EEPRFDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGTGWA
 51  NTVEPLTGIT ERVGRIWGVV SHLNCVADTP ELRAVYNELM PEITVFFTEI
101  GQDIELYNRF KTIKNSPEFD TLSPAQKTKL NH
//
  1  YASEKLREAK YAFSETXVKK YFPVGXVLNG LFAQXKKLYG IGFTEKTPVP

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```

51  WHKDVRYXEL QQNGEXIGGV YMDLYAREGK RGGAWMNDYK GRRRFSDGTL
101 QLPTAYLVCN FAPPVGGREA RLSHDEILIL FHETGHGLHH LLTQVDELGV
151 SGINGVXWDA VELPSQFMEN FVWEYNVLAQ XSAHEETGVP LPKELXDKXL
201 AAKNFQXGMF XVRQXEFALF DMMIYSEDDE GRLKNWQQVL DSVRKKVAVI
251 QPPEYNRFAL SFGHIFAGGY SAAXYSYAWA EVLSADAYAA FEESDDVAAT
301 GKRFWQEILA VGXSRSGAES FKAFRGREPS IDALLRHS GF DNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 52>:

```

g128.seq
1  atgattgaca acgCActgct ccacttgggc gaagaaccCC GTTTTaatca
51  aatccaaacc gaagACAtca AACCCGCCGT CCAAACCGCC ATCGCCGAAG
101 CGCGCGGACA AATCGCCGCC GTCAAAGCGC AAACGCACAC CGGCTGGGCG
151 AACACCGTCG AGCGTCTGAC CGGCATCACC GAACGCGTCG GCAGGATTG
201 GGGCGTCGTG TCCCATCTCA ACTCCGTCGT CGACACGCCG GAAGTGCAGC
251 CCGTCTATAA CGAACTGATG CCTGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTGCA ACGCTTTCCT CCGCACAAAA AACCAAGCTC GATCACGACC
401 TGCGCGATTT CGTATTGAGC GGCGCGGAAC TGCCGCCCGA ACGGCAGGCA
451 GAACTGGCAA AACTGCAAAAC CGAAGGCGCG CAACTTTCCG CCAAATTCTC
501 CCAAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCGCT TGCCGCGATT CCCGAAGACG CGCTCGCCAT GTTTGCCGCC
601 GCCGCGCAAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCAGATTCC
651 GCACTACCTT GCCGTATACC AATACGCCGG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGTGCCA GCGAACTTTC AAACGACGGC
751 AAATTCGACA ACACCGCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801 AACCGccaaa cTGCTCGGCT TTAAAAATTA CGCCGAATTG TCGCTGGCAA
851 CCAAAATGGC GGACACGCCG GAACAGGTTT TAAACTTCCT GCACGACCTC
901 GCCCGCCGCG CCAAACCTTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTCGCCCGC GAACACCTCG GTCTCGCCGA CCCGCAGCCG TGGGACTTGA
1001 GCTACGCCCG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAAA GTTCTGGCAG GCCTGTTCCG
1101 CCAAATCAAA AAATCTACG GCATCGGATT CGCCGAAAAA ACCGTTCCCC
1151 TCTGGCACAA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCAAAACC
1201 ATCGGCGGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACGCGGCGG
1251 CGCGTGGATG AACGACTaca AAGGCCGCCG CCGCTTTGCC GACGgcacGC
1301 TGCAACTGCC CACCGCTTAC CTGCTTGCA ACTTCGCCCC GCCCGTCGGC
1351 GGCAAGAAG CGCGTTAAG CCACGACGAA ATCCTCACCC TCTTCCACGA
1401 AacCGGCCAC GGAATGCACC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG
1451 TGTCGGGCAT CAacggcgtA GAATGGGACG CGGTCGAACT GCCAGCCAG
1501 TTTATGGAAA ACTTCGTTTG GGAATACAAT GTATTGGCAC AAATGTCGCG
1551 CCACGAAGAA AccgGCGAGC CCCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TcgcCGCCAA AAATTTCCAG CGCGGTATGT TCCTCGTCCG GCAAATGGAG
1651 TTCGCCCTCT TCGATATGAT GATTTACAGT GAAAGCGACG AATGCCGTCT
1701 GAAAAACTGG CAGCAGGTTT TAGACAGCGT GCGCAAAGAA GTcGCCGTCA
1751 TCCAACCGCC CGAATACAAC CGCTTCGCCA ACAGCTTCGG CCacatctTC
1801 GCcggcGGCT ATTCCGCAGG CTATTACAGC TACGCATGGG CCGAAGTCct
1851 cAGCACCGAT GCCTACGCCG CCTTTGAAGA AAGcGACGac gtcGCCGCCA
1901 CAGGCAAACG CTTCTGGCAA GAAAtccttg ccgtcggcgg ctCCCGCAGC
1951 gcgGCGGAAT CCTTCAAAGC CTTCCGCGGA CGCGAACC GAATAGACGC
2001 ACTGCTGCGC CAaagcggtT TCGACAACGC gGCTtgA

```

This corresponds to the amino acid sequence <SEQ ID 53; ORF 128.ng>:

```

g128.pep
1  MIDNALLHLG EEPRFNQIQT EDIKPAVQTA IAEARGQIAA VKAQHTGWGA
51  NTVERLTGIT ERVGRIGWV SHLNSVVDTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKGY KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG

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```

251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDL SYAGEKL REAKYAFSET
351 EVKKYFPVGK VLAGLFAQIK KLYGIGFAEK TVPVWHKDVR YFELQONGKT
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTLLQLPTAY LVCNFAPPVG
451 GKEARLSHDE ILTLFHETGH GLHHLLTQVD ELGVSGINGV EWD AVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGEPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDDMIYS ESDECRLKNW QQVLDSVRKE VAVIQPPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSTD AYAAFEESDD VAATGKRFWQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR QSGFDNAA*

```

ORF 128 shows 91.7% identity over a 475 aa overlap with a predicted ORF (ORF 128.ng) from *N. gonorrhoeae*:

m128/g128

	10	20	30	40	50	60
g128.pep	MIDNALLHLGEEPFRNQIQTEDIKPAVQTAIAEARGQIAAVKAQTHTGWANTVERLTGIT					
	: : :					
m128	MTDNALLHLGEEPFRDQIKTEDIKPALQTAIAEAREQIAAIKAQTHTGWANTVEPLTGIT					
	10	20	30	40	50	60
	70	80	90	100	110	120
g128.pep	ERVGRIWGVVSHLNSVVDTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA					
	: : :					
m128	ERVGRIWGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
	70	80	90	100	110	120
	130	140	150	160	170	180
g128.pep	TLSPAQKTKLDHDLRDFVLSGAELPPERQAEALAKLQTEGAQLSAKFSQNVLDATDAFGIY					
	:					
m128	TLSPAQKTKLNH					
	130					
	//					
				340	350	360
g128.pep	YAGEKLREAKYAFSETEVKKYFPVGKVLG					
	:					
m128	YASEKLREAKYAFSETXVKKYFPVGXVLNG					
				10	20	30
	370	380	390	400	410	420
g128.pep	LFAQIKKLYGIGFAEKTVPVWHKDVR YFELQONGKTIGGVYMDLYAREGKRGGAWMNDYK					
	: : :					
m128	LFAQXKKLYGIGFTEKTVPVWHKDVR YXELQONGEXIGGVYMDLYAREGKRGGAWMNDYK					
	40	50	60	70	80	90
	430	440	450	460	470	480
g128.pep	GRRRFADGTLQLPTAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHHLLTQVDELGV					
	: : : :					
m128	GRRRFSDGTLQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHHLLTQVDELGV					
	100	110	120	130	140	150
	490	500	510	520	530	540
g128.pep	SGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGEPLPKELFDKMLAAKNFQRMGMF					
m128	SGINGVXWDAVELPSQFMENFVWEYNVLAQXSAHEETGVPLPKELXDKXLAAKNFQXGMF					
	160	170	180	190	200	210
	550	560	570	580	590	600

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g128.pep	LVRQMEFALFDMMIYSESEDECRLKNWQQVLDSVRKEVAVIQPPEYNRFANSFGHIFAGGY
	: :
m128	XVRQXEFALFDMMIYSEDEDEGR LKNWQQVLDSVRKKVAVIQPPEYNRFALSFGHIFAGGY
	220 230 240 250 260 270
g128.pep	610 620 630 640 650 660
	SAGYYSYAWAEVLSTDAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRGREPS
	: :
m128	SAAXYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGXSRSGAESFKAFRGREPS
	280 290 300 310 320 330
g128.pep	670 679
	IDALLRQSGFDNAAX
	: :
m128	IDALLRHSGFDNAVX
	340

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 54>:

```

a128.seq
1  ATGACTGACA ACGCACTGCT CCATTTGGGC GAAGAACCCC GTTTTGATCA
51  AATCAAAACC GAAGACATCA AACCCGCCCT GCAAACCGCC ATTGCCGAAG
101 CGCGCGAACA AATCGCCGCC ATCAAAGCCC AAACGCACAC CGGCTGGGCA
151 AACACTGTCTG AACCCCTGAC CGGCATCACC GAACGCGTCG GCAGGATTTG
201 GGGCGTGGTG TCGCACCTCA ACTCCGTACG CGACACGCCG GAACTGCGCG
251 CCGCTACAA TGAATTAATG CCCGAAATTA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAGCTGTA CAACCGCTTC AAAACCATCA AAAACTCCCC
351 CGAGTTCGAC ACCCTCTCCC ACGCGCAAAA AACCAAATC AACCACGATC
401 TGCGCGATTT CGTCCTCAGC GCGCGCGAAC TGCCGCCCCG ACAGCAGGCA
451 GAATTGGCAA AACTGCAAAAC CGAAGGCGCG CAACTTTCCG CCAAATTCCTC
501 CCAAACGTC CTAGACGCGA CCGACGCGTT CCGCATTTAC TTTGACGATG
551 CCGCACCGCT TGCCGGCATT CCCGAAGACG CGCTCGCCAT GTTTGCCGCT
601 GCCGCGCAAA GCGAAGGCAA AACAGGTAC AAAATCGGTT TGCAGATTCC
651 GCACTACCTC GCCGTCATCC AATACGCCGA CAACCGCAAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGCGCCA GCGAGCTTTC AGACGACGGC
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGAAA ACGCCCTGCA
801 AACCGCCAAA CTGCTCGGCT TCAAAAATA CGCCGAATTG TCGCTGGCAA
851 CCAAATGGC GGACACCCCC GAACAAGTTT TAAACTTCCT GCACGACCTC
901 GCGCGCCGCG CCAAACCCTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTCGCCCCG GAAAGCCTCG GCCTCGCCGA TTTGCAACCG TGGGACTTGG
1001 GCTACGCCGG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAAA GTATTAAACG GACTGTTCCG
1101 CCAAATCAAA AAATCTACG GCATCGGATT TACCGAAAAA ACCGTCCCCG
1151 TCTGGCACA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCGAAACC
1201 ATAGCGGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACCGCGCGG
1251 CGCGTGGATG AACGACTACA AAGGCCGCCG CCGTTTTTCA GACGGCACGC
1301 TGCAACTGCC CACCGCTTAC CTCGTCTGCA ACTTCACCCC GCCCGTCGGC
1351 GGCAAGAAG CCCGCTTGAG CCATGACGAA ATCCTCACCC TCTTCCACGA
1401 AACCAGACAC GGCCTGCACC ACCTGCTTAC CCAAGTCGAC GAACTGGGCG
1451 TATCCGGCAT CAACGGCGTA GAATGGGACG CAGTCGAACT GCCAGTCAG
1501 TTTATGGAAA ATTTCTGTTG GGAATACAAT GTCTTGGCGC AAATGTCCGC
1551 CCACGAAGAA ACCGGCGTTC CCCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TCGCCGCCAA AAATTTCCAA CGCGGAATGT TCCTCGTCCG CCAAATGGAG
1651 TTCGCCCTCT TTGATATGAT GATTTACAGC GAAGACGACG AAGGCCGTCT
1701 GAAAACTGG CAACAGGTTT TAGACAGCGT GCGCAAAGAA GTCGCCGTCG
1751 TCCGACCGCC CGAATACAAC CGCTTCGCCA ACAGCTTCGG CCACATCTTC
1801 GCAGGCGGCT ATTCCGAGG CTATTACAGC TACGCGTGGG CGGAAGTATT
1851 GAGCGCGGAC GCATACGCCG CCTTTGAAGA AAGCGACGAT GTCGCCGCCA
1901 CAGGCAAACG CTTTTGGCAG GAAATCCTCG CCGTCGGCGG ATCGCGCAGC
1951 GCGGCAGAAT CCTTCAAAGC CTTCCGCGGA CGCGAACCGA GCATAGACGC
2001 ACTCTTGCGC CACAGCGGCT TCGACAACGC GGCTTGA

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This corresponds to the amino acid sequence <SEQ ID 55; ORF 128.a>:

```

a128.pep
  1 MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGTGWA
 51 NTVEPLTGIT ERVGRIWGVV SHLNSVTDTP ELRAAYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSHAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYADNRK LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLENALQTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR ESLGLADLQP WDLGYAGEKL REAKYAFSET
351 EVKKYFPVGK VLNGLFAQIK KLYGIGFTEK TVPVWHKDVR YFELQQNGET
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFS DGTQLQPTAY LVCNFTPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV EWDAVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS EDDEGRKLNW QQVLDSVRKE VAVVRPPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAAFEESDD VAATGKRWFQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR HSGFDNAA*

```

m128/a128 ORFs 128 and 128.a showed a 66.0% identity in 677 aa overlap

```

              10      20      30      40      50      60
m128.pep      MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQHTGTGWANTVEPLTGIT
              |||
a128           MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQHTGTGWANTVEPLTGIT
              10      20      30      40      50      60

              70      80      90      100     110     120
m128.pep      ERVGRIWGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
              |||
a128           ERVGRIWGVVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
              70      80      90      100     110     120

              130
m128.pep      TLSPAQKTKLNH-----
              |||
a128           TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY
              130      140      150      160      170      180

m128.pep      -----
a128           FDDAAPLAGIPEDALAMFAAAAQSEGKTGYKIGLQIPHYLAVIQYADNRKLREQIYRAYV
              190      200      210      220      230      240

m128.pep      -----
a128           TRASELSDDGKFDNTANIDRTLENALQTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL
              250      260      270      280      290      300

              140      150
m128.pep      -----YASEKLREAKYAFSETXVKKYFPVGX
              |||
a128           ARRAKPYAEKDLAEVKAFARESLGLADLQPWDLGYAGEKLREAKYAFSETEVKKYFPVGK
              310      320      330      340      350      360

              160      170      180      190      200      210
m128.pep      VLNGLFAQXKKLYGIGFTEKTVPVWHKDVRXYELQQNGEXIGGVYMDLYAREGKRGGAWM
              |||
a128           VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRXYFELQQNGETIGGVYMDLYAREGKRGGAWM
              370      380      390      400      410      420

              220      230      240      250      260      270
m128.pep      NDYKGRRRFSDGTQLQPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVD

```

a128	NDYKGRRRFS	SDGTLQLPTAYLVCNFT	PPVGGKEARLSHDEIL	TLFHETGHGLHLLTQVD	
	430	440	450	460	470 480
m128.pep	280	290	300	310	320 330
	ELGVSGINGVXWDAVELPSQFMENFVWEYNVLAQXSAHEETGVPLPKELXDKXLAAKNFQ				
a128	ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ				
	490	500	510	520	530 540
m128.pep	340	350	360	370	380 390
	XGMFXVRQXEFALFDMMIYSEDDEGR LKNWQQVLDSVRKKVAVIQPPEYNRFALSFGHIF				
a128	RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQQVLDSVRKEVAVVRPEYNRFANSFGHIF				
	550	560	570	580	590 600
m128.pep	400	410	420	430	440 450
	AGGYSAAXYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGXSRSGAESFKAFRG				
a128	AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRG				
	610	620	630	640	650 660
m128.pep	460	470			
	REPSIDALLRHSGFDNAVX				
a128	REPSIDALLRHSGFDNAAX				
	670				

m128-1.seq

1	ATGACTGACA	ACGCACTGCT	CCATTTGGGC	GAAGAACCCC	GTTTGTATCA
51	AATCAAAACC	GAAGACATCA	AACCCGCCCT	GCAAACCGCC	ATCGCCGAAG
101	CGCGCGAACA	AATCGCCGCC	ATCAAAGCCC	AAACGCACAC	CGGCTGGGCA
151	AACACTGTGC	AACCCCTGAC	CGGCATCACC	GAACGCGTCG	GCAGGATTTG
201	GGGCGTGGTG	TCGCACCTCA	ACTCCGTGCG	GCACACGCCC	GCAATGCGCG
251	CCGTCTATAA	CGAACTGATG	CCCGAAATCA	CCGTCTTCTT	CACCGAAATC
301	GGACAAGACA	TCGAGCTGTA	CAACCGCTTC	AAAACCATCA	AAAATTCCCC
351	CGAATTTCGAC	ACCCCTCTCC	CCGCACAAA	AACCAAATCA	AACCACGATC
401	TGCGCGATTT	CGTCTCTAGC	GGCGCGGAAC	TGCCGCCGCA	ACAGCAGGCA
451	GAAGTGGCAA	AACTGCAAAC	CGAAGGCGCG	CAACTTTCCG	CCAAATTCTC
501	CCAAAACGTC	CTAGACGCGA	CCGACGCGTT	CGGCATTTAC	TTTGACGATG
551	CCGCACCGCT	TGCCGGGCAT	CCCGAAGAGC	CGCTCGCCAT	GTTTGCCGCC
601	GCCGCGCAAA	GCGAAAGCAA	AACAGGCTAC	AAAATCGGCT	TGCAGATTCC
651	ACACTACCTC	CGCGTCAATC	AATACGCCGA	CAACCGCGAA	CTGCGGCAAC
701	AAATCTACCG	CGCCTACGTT	ACCCGCGCCA	CGGAAC TTTC	AGACGACGGC
751	AAATTGACAC	ACACCGCCAA	CATCGACCGC	ACGCTCGCAA	ACGCCCTGCA
801	AACCGCCAAA	CTGCTCGGCT	TCAAAAAC TA	CGCCGAATTG	TCGCTGGCAA
851	CCAAAATGGC	GGACACGCCC	GAACAAGTTT	TAAACTTCTT	GCACGACCTC
901	GCCCGCCGCG	CCAAACCC TA	CGCCGAAAAA	GACCTCGCCG	AAGTCAAAGC
951	CTTCGCCCGC	GAAAGCCTGA	ACCTCGCCGA	TTTGCAACCG	TGGGACTTGG
1001	GCTACGCCAG	CGAAAACTG	CGCGAAGCCA	AATACGCGTT	CAGCGAAACC
1051	GAAGTCAAAA	AATACTTCCC	CGTCGGGCAA	GTATTAAACG	GACTGTTTCG
1101	CCAAATCAAA	AAACTCTACG	GCATCGGAAT	TACCGAAAAA	ACCGTCCCCG
1151	TCTGGCACAA	AGACGTGCGC	TATTTTGAAT	TGCAACAAAA	CGCGGAAACC
1201	ATAGGCGGCG	TTTATATGGA	TTTGTACGCA	CGCGAAGGCA	AACGCGGCGG
1251	CGCGTGGATG	AACGACTACA	AAGGCCGCGG	CGTTTTTTCA	GACGGCACGC
1301	TGCAACTGCC	CACCGCCTAC	CTCTGTCTGA	ACTTCGCCCC	ACCGTTCGGC
1351	GGCAGGGGAC	CCCGCCTGAC	CCACGACGAA	ATCTCATCTC	TCTTCCACGA
1401	AACCGGACAC	GGGCTGCACC	ACCTGCTTAC	CCAAGTGGAC	GAACTGGGCG

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1451 TATCCGGCAT CAACGGCGTA GAATGGGACG CGGTGGAAC T GCCCAGCCAG
1501 TTTTATGGAAA ATTTCTGTTT GGAATACAAT GTCTTGGCAC AAATGTCAGC
1551 CCACGAAGAA ACCGGCGTTC CCCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TCGCCGCCAA AAACCTCCAA CGCGGCATGT TCCTCGTCCG GCAAATGGAG
1651 TTCGCCCTCT TTGATATGAT GATTTACAGC GAAGACGACG AAGGCCGTCT
1701 GAAAAACTGG CAACAGGTTT TAGACAGCGT GCGCAAAAA GTCGCCGTCA
1751 TCCAGCCGCC CGAATACAAC CGCTTCGCCT TGAGCTTCGG CCACATCTTC
1801 GCAGGCGGCT ATTCGCGAG CTATTACAGC TACGCGTGGG CGGAAGTATT
1851 GAGCGCGGAC GCATACGCCG CCTTTGAAGA AAGCGACGAT GTCGCCGCCA
1901 CAGGCAAACG CTTTTGGCAG GAAATCCTCG CCGTCGGCGG ATCGCGCAGC
1951 GCGGCAGAAT CTTCAAAGC CTTCCGCGGC CGCGAACC GAATAGACGC
2001 ACTCTTGC GC CACAGCGGTT TCGACAACGC GGTCTGA

```

This corresponds to the amino acid sequence <SEQ ID 57; ORF 128-1>:

m128-1.pcp.

```

1 MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGTGWA
51 NTVEPLTGIT ERVGRWGVV SHLNSVADTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSPAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSESKTGY KIGLQIPHLY AVIQYADNRE LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLANALQTAK LLGFKNYAE SLATKMADTP EQVLNLFHDL
301 ARRAKPYAEK DLAEVKAFAR ESLNLADLQ WDLGYASEKL REAKYAFSET
351 EVKKYFPVGK VLNLGFAQIK KLYGIGFTEK TVPVVHKDVR YFELQONGET
401 IGVVYMDLYA REGKRGGAWM NDYKGRRRFS DGTLLQLPTAY LVCNFAPPVG
451 GREARLSHDE ILILFHETGH GLHLLLTQVD ELGVSGINGV EWDAVEPLSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS EDDEGRLKNW QQVLDSVRKK VAVIQPPEYN RFALSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAFAEESDD VAATGKRFWQ EILAVGGSRS
651 AESFKAFRG REPSIDALLR HSGFDNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 58>:

g128-1.seq (partial)

```

1 ATGATTGACA ACGCACTGCT CCACTTGGGC GAAGAACCCC GTTTTAATCA
51 AATCAAAACC GAAGACATCA AACC CGCGT CCAAACCGCC ATCGCCGAAG
101 CGCGCGGACA AATCGCCGCC GTCAAAGCGC AAACGCACAC CGGCTGGCGG
151 AACACCGTCG AGCGTCTGAC CGGCATCACC GAACGCGTCG GCAGGATTTG
201 GGGCGTCGTG TCCCATCTCA ACTCCGTCTG CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCTGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTTGCA ACGCTTCC CCGCACAAAA AACCAGCTC GATCAGACC
401 TGCGCGATTT CGTATTGAGC GGCGCGGAAC TGCCGCCCGA ACGGCAGGCA
451 GAACTGGCAA AACTGCAAAC CGAAGGCGCG CAACTTTCCG CCAAATTCTC
501 CCAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCGCT TGCCGGCATT CCCGAAGACG CGCTCGCCAT GTTTGCCGCC
601 GCGCGCAAAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCAGATTCC
651 GCACTACCTT GCCGTTATCC AATACGCCG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCTACGTT ACCCGTGCCA GCGAACTTTC AAACGACGGC
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801 AACCGCCAAA CTGCTCGGCT TTAAAAATTA CGCCGAATTG TCGCTGGCAA
851 CCAAATGGC GGACACGCCC GAACAGGTTT TAAACTTCCT GCACGACCTC
901 GCGCGCGCG CCAAACCTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTGCGCCGC GAACACCTCG GTCTCGCCGA CCCGACGCCG TGGGACTTGA
1001 GTACGCGCG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAA GTTCTGGCAG GCCTGTTCCG
1101 CCAATCAAAA AAACCTACG GCATCGGATT CGCCGAAAAA ACCGTTCGCC
1151 TCTGGCACA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCAAAACC
1201 ATCGCGGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACGCGGCGG
1251 CGCGTGATG AACGACTACA AAGGCCGCC CCGCTTTGCC GACGGCACGC
1301 TGCAACTGCC CACCGCTAC CTCGTCTGCA ACTTCGCCCC GCCCGTCGGC
1351 GGCAAGAAG CGCGTTAAG CCACGACGAA ATCCTCACCC TCTTCCACGA
1401 AACC GGCCAC GACTGCACC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG
1451 TGTCGGCAT CAACGGCGTA AAA

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This corresponds to the amino acid sequence <SEQ ID 59; ORF 128-1.ng>:

g128-1.pep (partial)
 1 MIDNALLHLG EEPFRNQIKT EDIKPAVQTA IAEARGQIAA VKAQHTGTGWA
 51 NTVERLTGIT ERVGRIWGVV SHLNSVVDTP ELRAVYNELM PEITVFFTEI
 101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA
 151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
 201 AAQSEGKTGY KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG
 251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
 301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDLSYAGEKL REAKYAFSET
 351 EVKKYFPVGK VLAGLFAQIK KLYGIGFAEK TVPVVHKDVR YFELQONGKT
 401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTQLQLPTAY LVCNFAPPVG
 451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV K

m128-1/g128-1 ORFs 128-1 and 128-1.ng showed a 94.5% identity in 491 aa overlap

g128-1.pep	10	20	30	40	50	60
	MIDNALLHLGEEPRFNQIKTEDIKPAVQTAIAEARGQIAAVKQHTGTGWANTVERLTGIT					
m128-1	10	20	30	40	50	60
	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIAKQHTGTGWANTVEPLTGIT					
g128-1.pep	70	80	90	100	110	120
	ERVGRIWGVVSHLNSVVDTPPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA					
m128-1	70	80	90	100	110	120
	ERVGRIWGVVSHLNSVADTPPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
g128-1.pep	130	140	150	160	170	180
	TLSPAQKTKLDHDLRDFVLSGAELPPERQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	130	140	150	160	170	180
	TLSPAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
g128-1.pep	190	200	210	220	230	240
	FDDAAPLAGIPEDALAMFAAAAQSEGKTGYKIGLQIPHYLAVIQYAGNRELREQIYRAYV					
m128-1	190	200	210	220	230	240
	FDDAAPLAGIPEDALAMFAAAAQSEKSGTYKIGLQIPHYLAVIQYADNRELREQIYRAYV					
g128-1.pep	250	260	270	280	290	300
	TRASELSNDGKFDNTANIDRTLLENALKTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL					
m128-1	250	260	270	280	290	300
	TRASELSDDGKFDNTANIDRTLANALQTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL					
g128-1.pep	310	320	330	340	350	360
	ARRAKPYAEKDLAEVKAFAREHLGLADPQPWDLSYAGEKLREAKYAFSETEVKKYFPVGK					
m128-1	310	320	330	340	350	360
	ARRAKPYAEKDLAEVKAFARESLNLADLPWDLGYASEKLREAKYAFSETEVKKYFPVGK					
g128-1.pep	370	380	390	400	410	420
	VLAGLFAQIKKLYGIGFAEKTVPVWHKDVRYFELQONGKTIGGVYMDLYAREGKRGGAWM					
m128-1	370	380	390	400	410	420
	VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRYFELQONGKTIGGVYMDLYAREGKRGGAWM					
g128-1.pep	430	440	450	460	470	480
	NDYKGRRRFADGTQLQLPTAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHLLTQVD					

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|||||||:|||||||:|||||||
m128-1  NDYKGRRRFSDGTLQLPTAYLVCNFPAPPVGGREARLSHDEILILFHETGHGLHLLTQVD
          430      440      450      460      470      480

          490
g128-1.pep  ELGVSGINGVK
          |||||:
m128-1  ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFO
          490      500      510      520      530      540

```

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 60>:

```

a128-1.seq
1  ATGACTGACA ACGCACTGCT CCATTTGGGC GAAGAACCCC GTTTTGATCA
51  AATCAAAACC GAAGACATCA AACCCGCCCT GCAAACCGCC ATTGCCGAAG
101 CGCGCGAACA AATCGCCGCC ATCAAAGCCC AAACGCACAC CGGCTGGGCA
151 AACACTGTCTG AACCCCTGAC CGGCATCACC GAACGCGTCG GCAGGATTTG
201 GGGCGTGGTG TCGCACCTCA ACTCCGTACG CGACACGCCC GAACTGCGCG
251 CCGCTACAA TGAATTAATG CCCGAAATTA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAGCTGTA CAACCGCTTC AAAACCATCA AAAACTCCCC
351 CGAGTTTCGAC ACCCTCTCCC ACGCGCAAAA AACCAAACTC AACCACGATC
401 TCGCGGATTT CGTCCTCAGC GCGCGGAAC TGCCGCCCGA ACAGCAGGCA
451 GAATTGGCAA AACTGCAAAC CGAAGGCGCG CAACTTTCGG CCAAATTCTC
501 CCAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCGCT TGCCGGCATT CCCGAAGACG CGCTCGCCAT GTTTGCCGCT
601 GCGCGCAAAA GCGAAGGCAA AACAGGCTAC AAAATCGGTT TGCAGATTCC
651 GCACTACCTC GCCGTCATCC AATACGCCGA CAACCGCAAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGCGCCA GCGAGCTTTC AGACGACGGC
751 AAATTCGACA ACACCGCAA CATCGACCGC ACGCTCGAAA ACGCCCTGCA
801 AACCGCAAAA CTGCTCGGCT TCAAAAAC TA CGCCGAATTG TCGCTGGCAA
851 CCAAATGGC GGACACCCCC GAACAAGTTT TAAACTTCCT GCACGACCTC
901 GCGCGCGCG CCAAACCCTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTGCGCCGC GAAAGCCTCG GCCTCGCCGA TTTGCAACCG TGGGACTTGG
1001 GCTACGCCGG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAAA GTATTAAACG GACTGTTTCG
1101 CCAAATCAAA AACTCTACG GCATCGGATT TACCGAAAAA ACCGTCCCCG
1151 TCTGGCACA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCGAAACC
1201 ATAGCGGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACGCGGCGG
1251 CCGTGATG AACGACTACA AAGGCCGCG CCGTTTTTCA GACGGCACGC
1301 TGCAACTGCC CACCGCTAC CTCGTCTGCA ACTTCAACCC GCCGTGCGG
1351 GGCAAGAAG CCGCTTGAG CCATGACGAA ATCCTACCC TCTTCCACGA
1401 AACCGGACAC GGCCTGCACC ACCTGCTTAC CCAAGTCGAC GAACTGGGCG
1451 TATCCGGCAT CAACGCGTA GAATGGGACG CAGTCGAACT GCCCAGTCAG
1501 TTTATGAAA ATTCGTTTG GGAATACAAT GTCTTGGCGC AAATGTCCGC
1551 CCACGAAGAA ACCGCGGTT CCCTGCCGAA AGAACTCTC GACAAAAATG
1601 TCGCGCCAA AACTTCCAA CGCGGAATGT TCCTCGTCCG CCAAATGGAG
1651 TTCGCCCTCT TTGATATGAT GATTACAGC GAAGACGACG AAGGCCGTCT
1701 GAAAACTGG CAACAGGTTT TAGACAGCGT GCGCAAAGAA GTCGCCGTCT
1751 TCCGACCGCC CGAATACAAC CGCTTCGCCA ACAGCTTCGG CCACATCTTC
1801 GCAGGCGGCT ATTCCGACG CTATTACAGC TACGCGTGGG CGGAAGTATT
1851 GAGCGCGGAC GCATACGCG CTTTGAAGA AAGCGACGAT GTCGCCGCCA
1901 CAGGCAAACG CTTTGGCAG GAAATCCTCG CCGTCGGCGG ATCGCGCAGC
1951 GCGGCAGAAT CTTCAAAGC CTTCCGCGGA CGCAACCGA GCATAGACGC
2001 ACTCTTGCGC CACAGCGGCT TCGACAACGC GGCTTGA

```

This corresponds to the amino acid sequence <SEQ ID 61; ORF 128-1.a>:

```

a128-1.pep
1  MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTHTGWA
51  NTVEPLTGIT ERVGRIWGVV SHLNSVTDTP ELRAAYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSHAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYADNRK LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLENALQTAK LLGFKNYAEL SLATKMADTP EQVLNLFHDL

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```

301  ARRAKPYAEK  DLAEVKAFAR  ESLGLADLQP  WDLGYAGEKL  REAKYAFSET
351  EVKKYFPVGK  VLNGLFAQIK  KLYGIGFTEK  TVPVWHKDVR  YFELQQNGET
401  IGGVYMDLYA  REGKRGGAWM  NDYKGRRRFS  DGTLLQLPTAY  LVCNFTPPVG
451  GKEARLSHDE  ILTLFHETGH  GLHLLLTQVD  ELGVSGINGV  EWDAVELPSQ
501  FMENFVWEYN  VLAQMSAHEE  TGVPLPKELF  DKMLAAKNFQ  RGMFLVRQME
551  FALFDMMIYS  EDDEGRLEKN  QQVLDSVRKE  VAVVRPPEYN  RFANSFGHIF
601  AGGYSAGYYS  YAWAEVLSAD  AYAAFEESDD  VAATGKRWFQ  EILAVGGSRS
651  AAESFKAFRG  REPSIDALLR  HSGFDNAA*

```

m128-1/a128-1 ORFs 128-1 and 128-1.a showed a 97.8% identity in 677 aa overlap

	10	20	30	40	50	60
a128-1.pep	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQTHTGWANTVEPLTGIT					
m128-1	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQTHTGWANTVEPLTGIT					
	10	20	30	40	50	60
	70	80	90	100	110	120
a128-1.pep	ERVGRIWGVVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
m128-1	ERVGRIWGVVSHLNSVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
	70	80	90	100	110	120
	130	140	150	160	170	180
a128-1.pep	TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	TLSPAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
	130	140	150	160	170	180
	190	200	210	220	230	240
a128-1.pep	FDDAAPLAGIPEDALAMFAAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKLREQIYRAYV					
m128-1	FDDAAPLAGIPEDALAMFAAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKLREQIYRAYV					
	190	200	210	220	230	240
	250	260	270	280	290	300
a128-1.pep	TRASELSDDGKFDNTANIDRTLENALQTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL					
m128-1	TRASELSDDGKFDNTANIDRTLENALQTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL					
	250	260	270	280	290	300
	310	320	330	340	350	360
a128-1.pep	ARRAKPYAEKDLAEVKAFARESLGLADLQPWDLYAGEKLREAKYAFSETEVKKYFPVGK					
m128-1	ARRAKPYAEKDLAEVKAFARESLNLADLQPWDLYAGEKLREAKYAFSETEVKKYFPVGK					
	310	320	330	340	350	360
	370	380	390	400	410	420
a128-1.pep	VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRIFYELQQNGETIGGVYMDLYAREGKRGGAWM					
m128-1	VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRIFYELQQNGETIGGVYMDLYAREGKRGGAWM					
	370	380	390	400	410	420
	430	440	450	460	470	480
a128-1.pep	NDYKGRRRFS DGTLLQLPTAYLVCNFTPPVGGKEARLSHDEILTLFHETGHGLHLLLTQVD					
m128-1	NDYKGRRRFS DGTLLQLPTAYLVCNFTPPVGGKEARLSHDEILTLFHETGHGLHLLLTQVD					
	430	440	450	460	470	480
	490	500	510	520	530	540
a128-1.pep	ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ					

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```

m128-1      ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ
              490      500      510      520      530      540

              550      560      570      580      590      600
a128-1.pep  RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQQVLD SVRKEVAVVRPPEYNRFANSFGHIF
              |||||||
m128-1      RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQQVLD SVRKKVAVIQPPEYNRFALSFGHIF
              550      560      570      580      590      600

              610      620      630      640      650      660
a128-1.pep  AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRG
              |||||||
m128-1      AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRG
              610      620      630      640      650      660

              670      679
a128-1.pep  REPSIDALLRHSGFDNAAX
              |||||||
m128-1      REPSIDALLRHSGFDNAVX
              670

```

206

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 62>:

```

m206.seq
1   ATGTTTCCCC CCGACAAAAC CCTTTTCCTC TGTCTCAGCG CACTGCTCCT
51  CGCCTCATGC GGCACGACCT CCGGCAAACA CCGCCAACCG AAACCCAAAC
101 AGACAGTCCG GCAAATCCAA GCCGTCCGCA TCAGCCACAT CGACCGCACA
151 CAAGGCTCGC AGGAACTCAT GCTCCACAGC CTCGGACTCA TCGGCACGCC
201 CTACAAATGG GGCGGCAGCA GCACCGCAAC CGGCTTCGAT TGCAGCGGCA
251 TGATTCAATT CGTTTACAAT AACGCCCTCA ACGTCAAGCT GCCGCGCACC
301 GCGCGCGACA TGGCGGCGGC AAGCCGSAAT ATCCCCGACA GCCGCTCAAT
351 GGCGGCGGAC CTCGTATTCT TCAACACCGG CGGCGCACAC CGCTACTCAC
401 ACGTCGGACT CTACATCGGC AACGGCGAAT TCATCCATGC CCCCAGCAGC
451 GGCAAAACCA TCAAAACCGA AAAACTCTCC ACACCGTTTT ACGCCAAAAA
501 CTACCTCGGC GCACATACTT TTTTACAGA ATGA

```

This corresponds to the amino acid sequence <SEQ ID 63; ORF 206>:

```

m206.pep..
1   MFPPDKTLFL CLSALLLASC GTTSGKHRQP KPKQTVRQIQ AVRISHIDRT
51  QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQFVYK NALNVKLPRP
101 ARDMAAASRK IPDSRXKAGD LVFFNTGGAH RYSHVGLYIG NGEFIHAPSS
151 GKTIKTEKLS TPFYAKNYLG AHTFFTE*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 64>:

```

g206.seq
1   atgttttccc ccgacaaaac ccttttcctc tgtctcggcg cactgctcct
51  cgctcatgac ggcacgacct ccggcaaaca ccgccaaccc aaacccaaac
101 agacagtccg gcaaattcaa gccgtccgca tcagccacat cggcgcgaca
151 caaggctcgc aggaactcat gctccacagc ctcggactca tcggcacgcc
201 ctacaaatgg ggcggcagca gcaccgcaac cggcttcgac tgcagcggca
251 tgattcaatt ggtttacaaa aacgccctca acgtcaagct gccgcgcacc
301 gccgcgcaca tggcggcggc aagccgcaaa atccccgaca gccgcctcaa
351 ggccggcgac atcgatttct tcaacacccg cggcgcacac cgctactcac
401 acgtcggact ctacatcggc aacggcggaat tcattccatgc ccccggcagc
451 ggcaaaacca tcaaaaccga aaaaactctc acaccgtttt acgccaaaaa
501 ctaccttgga gcgcatacgt tttttacaga atga

```

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This corresponds to the amino acid sequence <SEQ ID 65; ORF 206.ng>:

```

g206.pep
  1 MFSPDKTLFL CLGALLLASC GTTSGKHRQP KPKQTVRQIQ AVRISHIGRT
 51 QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQLVYK NALNVKLPR
101 ARDMAAASRK IPDSRLKAGD IVFFNTGGAH RYSHVGLYIG NGEFIHAPGS
151 GGTIKTEKLS TPFYAKNYLG AHTFFTE*

```

ORF 206 shows 96.0% identity over a 177 aa overlap with a predicted ORF (ORF 206.ng) from *N. gonorrhoeae*:

```

m206/g206

      10      20      30      40      50      60
m206.pep MFPPDKTLFLCLSALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
          |||||||:|||||
g206      MFSPDKTLFLCLGALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIGRTQGSQELMLHS
          10      20      30      40      50      60

      70      80      90     100     110     120
m206.pep LGLIGTPYKWGGSSTATGFD CSGMIQFVYK NALNVKLPR TARDMAAASRK IPDSRXKAGD
          |||||||:|||||
g206      LGLIGTPYKWGGSSTATGFD CSGMIQLVYK NALNVKLPR TARDMAAASRK IPDSRLKAGD
          70      80      90     100     110     120

      130     140     150     160     170
m206.pep LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX
          :|||||:|||||
g206      IVFFNTGGAHRYSHVGLYIGNGEFIHAPSGKTIKTEKLSTPFYAKNYLGAHTFFTE
          130     140     150     160     170

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 66>:

```

a206.seq
  1 ATGTTTCCCC CCGACAAAAC CCTTTTCCTC TGTCTCAGCG CACTGCTCCT
 51 CGCCTCATGC GGCACGACCT CCGGCAAACA CCGCCAACCG AAACCCAAAC
101 AGACAGTCCG GCAAATCCAA GCCGTCCGCA TCAGCCACAT CGACCGCACA
151 CAAGGCTCGC AGGAACTCAT GCTCCACAGC CTCGGACTCA TCGGCACGCC
201 CTACAAATGG GGCGGCAGCA GCACCGCAAC CGGCTTCGAT TGCAGCGGCA
251 TGATTCAATT CGTTACAAA AACGCCCTCA ACGTCAAGCT GCCGCGCACC
301 GCCCGCGACA TGGCGCGGCG AAGCCGCAAA ATCCCCGACA GCCGCCTTAA
351 GGCCGCGGAC CTCGTATTCT TCAACACCGG CGGCGCACAC CGCTACTCAC
401 ACGTCGGACT CTATATCGGC AACGGCGAAT TCATCCATGC CCCAGCAGC
451 GGCAAAACCA TCAAAACCGA AAAACTCTCC ACACCGTTT ACGCCAAAAA
501 CTACCTCGGC GCACATACTT TCTTTACAGA ATGA

```

This corresponds to the amino acid sequence <SEQ ID 67; ORF 206.a>:

```

a206.pep
  1 MFPPDKTLFL CLSALLLASC GTTSGKHRQP KPKQTVRQIQ AVRISHIDRT
 51 QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQFVYK NALNVKLPR
101 ARDMAAASRK IPDSRLKAGD LVFFNTGGAH RYSHVGLYIG NGEFIHAPSS
151 GGTIKTEKLS TPFYAKNYLG AHTFFTE*

```

m206/a206 ORFs 206 and 206.a showed a 99.4% identity in 177 aa overlap

```

      10      20      30      40      50      60
m206.pep MFPPDKTLFLCLSALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
          |||||||
a206      MFPPDKTLFLCLSALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
          10      20      30      40      50      60

```

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	70	80	90	100	110	120
m206.pep	LGLIGTPYKWGGSSTATGFDCSGMIQFVYKNALNVKLPR	TARDMAAASRKIPDSRXKAGD				
a206	LGLIGTPYKWGGSSTATGFDCSGMIQFVYKNALNVKLPR	TARDMAAASRKIPDSRLKAGD				
	70	80	90	100	110	120
	130	140	150	160	170	
m206.pep	LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX					
a206	LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX					
	130	140	150	160	170	

287

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 68>:

```

m287.seq
1   ATGTTTAAAC GCAGCGTAAT CGCAATGGCT TGTATTTTTC CCCTTTCAGC
51  CTGCGGGGGC GCGGGTGGCG GATCGCCCGA TGTCAAGTCG GCGGACACGC
101 TGTCAAAACC TGCCGCCCCC GTTGTTTCTG AAAAAGAGAC AGAGGCCAAAG
151 GAAGATGCGC CACAGGCAGG TTCTCAAGGA CAGGGCGCGC CATCCGCACA
201 AGGCAGTCAA GATATGGCGG CGGTTTCGGA AGAAAATACA GGCAATGGCG
251 GTGCGGTAAC AGCGGATAAT CCCAAAATG AAGACGAGGT GGCACAAAAT
301 GATATGCCGC AAAATGCCGC CGGTACAGAT AGTTCGACAC CGAATCACAC
351 CCCGATCCG AATATGCTTG CCGGAAATAT GGAAAATCAA GCAACGGATG
401 CCGGGGAATC GTCTCAGCCG GCAAACCAAC CGGATATGGC AAATGCGGCG
451 GACGGAATGC AGGGGGACGA TCCGTGCGCA GCGGGGCAAA ATGCCGGCAA
501 TACGCTGCC CAAGGTGCAA ATCAAGCCGG AAACAATCAA GCCGCCGGTT
551 CTTCAGATCC CATCCCCGCG TCAAACCTTG CACCTGCGAA TGGCGGTAGC
601 AATTTTGGAA GGGTTGATTT GGCTAATGGC GTTTTGATTG ACGGGCCGTC
651 GCAAAATATA ACGTTGACCC ACTGTAAGG CGATTCTTGT AGTGGCAATA
701 ATTTCTTGG TGAAGAAGTA CAGCTAAAAT CAGAATTGGA AAAATTAAGT
751 GATCAGACA AAATAAGTAA TTACAAGAAA GATGGGAAGA ATGATAAATT
801 TGTGCGTTTG GTTGCCGATA GTGTGCAGAT GAAGGGAATC AATCAATATA
851 TTATCTTTTA TAAACCTAAA CCCACTTCAT TTGCGCGATT TAGGCGTTCT
901 GCACGGTCGA GCGGTCGCT TCCGGCCGAG ATGCCGCTGA TTCCCGTCAA
951 TCAGCGGAT ACGCTGATT TCGATGGGGA AGCGGTCAGC CTGACGGGGC
1001 ATTCCGGCAA TATCTTCGCG CCCGAAGGGA ATTACCGGTA TCTGACTTAC
1051 GGGGCGGAAA AATTGCCCGG CGGATCGTAT GCCCTTCGTG TTCAAGGCCGA
1101 ACCGCAAAA GCGGAAATGC TTGCGGGCGC GGCCGTGTAC AACGGCGAAG
1151 TACTGCATTT CCATACGGAA AACGGCCGTC CGTACCCGAC CAGGGGCAGG
1201 TTTGCCGCAA AAGTCGATTT CGGCAGCAAA TCTGTGGACG GCATTATCGA
1251 CAGCGGCGAT GATTTCATA TGGGTACGCA AAAATTCAAA GCCGCCATCG
1301 ATGGAACCG CTTTAAGGGG ACTTGACGG AAAATGGCAG CGGGGATGTT
1351 TCCGAAAGT TTTACGGCCC GGCCGGCGAG GAAGTGGCGG GAAAATACAG
1401 CTATCGCCG ACAGATGCGG AAAAGGGCGG ATTCGGCGTG TTTGCCGGCA
1451 AAAAAGAGCA GGATTGA

```

This corresponds to the amino acid sequence <SEQ ID 69; ORF 287>:

```

m287.pep
1   MFKRSVIAMA CIFALSACGG GGGSPDVKS ADTLSPKPAAP VVSEKETEAQ
51  EDAPQAGSQG QGAPSAQGSQ DMAAVSEENT GNNGAVTADN PKNEDEVAQN
101 DMPQNAAGTD SSTPNHTPDP NMLAGNMENQ ATDAGESSQP ANQPDMAAAN
151 DGMQGDPSA GGQNAAGNTAA QGANQAGNNQ AAGSSDPIPA SNPAPANGGS
201 NFRVLDLANG VLIDGPSQNI TLTHCKGDSC SGNNFLDEEV QLKSEFEKLS
251 DADKISNYKK DGKNDKFVGL VADSVQMKGI NQYIIFYKPK PTSFARFRRS
301 ARSRRLPAE MPLIPVNQAD TLIVDGEAVS LTGHSNIFA PEGNYRYLTY

```

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351 GAEKLPGGSY ALRVQGEPAK GEMLAGAAVY NGEVLHFHTE NGRPYPTRGR
 401 FAAKVDFGSK SVDGIIDSGD DLHMGTOQKF AAIDGNGFKG TWTENGSGDV
 451 SGKFYGPAGE EVAGKYSYRP TDAEKGFGV FAGKKEQD*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 70>:

g287.seq
 1 atgtttaaac gcagtgtgat tgcaatggct tgtatttttc ccctttcagc
 51 ctgtgggggc ggcggtggcg gatcgcccga tgtcaagtcg gcggacacgc
 101 cgtcaaaacc ggccgcccc gttgtgtctg aaaatgccgg ggaaggggtg
 151 ctgccgaaag aaaagaaaga tgaggaggca gcgggcggtg cgccgcaagc
 201 cgatacgag gacgcaaccg ccggagaagg cagccaagat atggcggcag
 251 ttctgcgaga aaatacaggc aatggcggtg cggcaacaac ggacaacccc
 301 aaaaatgaag acgcgggggc gcaaaatgat atgcccgaat atgcgcgcga
 351 atccgcaaat caaacaggga acaaccaacc cgccggttct tcagattccg
 401 ccccgcgctc aaaccctgcc cctgcgaatg gcggtagcga ttttggaagg
 451 acgaacgtgg gcaattctgt tgtgattgac ggaccgtcgc aaaatataac
 501 gttgaccac tgtaaaggcg attcttgtaa tgggtgataat ttattggatg
 551 aagaagcacc gtcaaaatca gaatttgaat aattaagtga tgaagaaaaa
 601 attaaagcag ataaaaaaga cgagcaacgg gagaattttg tcggtttggt
 651 tgctgacagg gtaaaaaagg atggaactaa caaatatac atcttctata
 701 cggacaaacc acctactcgt tctgcacggt cgaggaggtc gcttcgggcc
 751 gagattccgc tgattcccgt caatcaggcc gatacgtga ttgtggatgg
 801 ggaagcggtc agcctgacgg ggcattccgg caatatcttc gcgccgaag
 851 ggaattaccg gtatctgact tacggggcgg aaaaattgcc cggcggtatc
 901 tatgccctcc gtgtgcaagg cgaaccgga aaaggcgaaa tgcttgttgg
 951 cacggccgtg tacaacggcg aagtgtgca tttccatatg gaaaacggcc
 1001 gtccgtaccc gtccggaggc aggtttgccg caaaagtcga tttcggcagc
 1051 aaatctgtgg acggcattat cgacagcggc gatgatttgc atatgggtac
 1101 gcaaaaattc aaagccgcca tcgatggaaa cggctttaag gggacttga
 1151 cggaaaatgg cggcggggat gtttcgggaa ggttttacgg cccggccggc
 1201 gaggaagtgg cgggaaaata cagctatcgc ccgacagatg ctgaaaaggg
 1251 cggattcggc gtgtttgccg gcaaaaaaga tcgggattga

This corresponds to the amino acid sequence <SEQ ID 71; ORF 287.ng>:

g287.pep
 1 MFKRSVIAMA CIFPLSACGG GGGGSPDVKS ADTPSKPAAP VVAENAGEGV
 51 LPKEKKDEEA AGGAPQADTQ DATAGEGSQD MAAVSAENTG NGGAATTDNP
 101 KNEDAGAQN MPQNAESAN QTGNNQAGS SDSAPASNPA PANGGSDFRG
 151 TNVGNVVID GPSQNTLTH CKGDSCNGDN LLDEEAPSKS EFEKLSDEEK
 201 IKRYKKDEQR ENFVGLVADR VKKDGNTKYI IFYTDKPPTR SARSRRLPA
 251 EIPLIPVQA DTLIVDGEAV SLTGHSGNIF APEGNYRYLT YGAELPGRS
 301 YALRVQGEPA KGEMLVGTAV YNGEVLHFHM ENGRPYPSG RFAAKVDFGS
 351 KSVDGIIDSG DDLHMGTOKF KAAIDGNGFK GTWTENGSGD VSGRFYGPAG
 401 EEVAGKYSYR PTDAEKGFGF VFAGKKDRD*

m287/g287 ORFs 287 and 287.ng showed a 70.1% identity in 499 aa overlap

	10	20	30	40	49
m287.pep	MFKRSVIAMACIFALSACGGGGGGSPDVKSADTLSPKPAAPVVSE-----KETE				
g287	MFKRSVIAMACIFPLSACGGGGGGSPDVKSADTPSKPAAPVVAENAGEGVLPKEKKDEEA				
	10	20	30	40	50
	50	60	70	80	90
m287.pep	KEDAPQAGSQGQAGPSAQGSQDMAVSEENTGNGGAVTADNPKNEDEVAQNMPQNAAGT				
g287	AGGAPQADTQD--ATAGEGSQDMAVSAENTGNGGAATTDNPKNEDAGAQNMPQNA--				
	70	80	90	100	110

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	110	120	130	140	150	160	169
m287.pep	DSSTPNHTPDPNMLAGNMENQATDAGESSQFANQPDMANAADGMQGGDDPSAGGQNAGNTA						
g287	-----						
	170	180	190	200	210	220	229
m287.pep	AQGANQAGNNQAAGSSDPIPASNPAPANGGSNFGRLVLANGLIDGPSQNTLTHCKGDS						
g287	-ESANQTGNQPAGSSDSAPASNPAPANGGSDFGRTNVGNVVIDGPSQNTLTHCKGDS						
	120	130	140	150	160	170	
	230	240	250	260	270	280	289
m287.pep	CSGNNFLDEEVQLKSEFEKLSADAKISNYKKDGKNDKFVGLVADSVQMKGINQYIIFYKP						
g287	CNGDNLLDEEAPSKSEFEKLSDEEKIKRYKKDEQRENFVGLVADRVKKDGTNKYIIFYTD						
	180	190	200	210	220	230	
	290	300	310	320	330	340	349
m287.pep	KPTSFARFRRSARSRRLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT						
g287	KPPT-----RSARSRRLPAEIPVNPQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT						
	240	250	260	270	280	290	
	350	360	370	380	390	400	409
m287.pep	YGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGS						
g287	YGAEKLPGGSYALRVQGEPAKGEMLVGTAVYNGEVLHFHMENGRPYPSGGRFAAKVDFGS						
	300	310	320	330	340	350	
	410	420	430	440	450	460	469
m287.pep	KSVDGIIDSGDDLHMGTOQKFKAAIDGNFGKGTWTENGSGDVSGKFYGPAGEEVAGKYSYR						
g287	KSVDGIIDSGDDLHMGTOQKFKAAIDGNFGKGTWTENGSGDVSGRFGYGPAGEEVAGKYSYR						
	360	370	380	390	400	410	
	470	480	489				
m287.pep	PTDAEKGGFGVFAGKKEQDX						
g287	PTDAEKGGFGVFAGKKDRDX						
	420	430					

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 72>:

```

a287.seq
1  ATGTTTAAAC GCAGTGTGAT TGCAATGGCT TGTATTGTTG CCCTTTCAGC
51  CTGTGGGGGC GGCGGTGGCG GATCGCCCGA TGTTAAGTCG GCGGACACGC
101 TGTCAAAACC TGCCGCCCTT GTTGTACTG AAGATGTCGG GGAAGAGGTG
151 CTGCCGAAAG AAAAGAAAGA TGAGGAGGCG GTGAGTGGTG CGCCGCAAGC
201 CGATACGCAG GACGCAACCG CCGGAAAAGG CGGTCAAGAT ATGGCGGCAG
251 TTTCGGCAGA AAATACAGGC AATGGCGGTG CGGCAACAAC GGATAATCCC
301 GAAAATAAAG ACGAGGGACC GCAAAATGAT ATGCCGCAAA ATGCCGCCGA
351 TACAGATAGT TCGACACCGA ATCACACCCC TGCACCGAAT ATGCCAACCA
401 GAGATATGGG AAACCAAGCA CCGGATGCCG GGGAATCGGC ACAACCGGCA
451 AACCAACCGG ATATGGCAAA TGCGGCGGAC GGAATGCAGG GGGACGATCC
501 GTCGGCAGGG GAAAATGCCG GCAATACGGC AGATCAAGCT GCAAATCAAG
551 CTGAAAACAA TCAAGTCGGC GGCTCTCAAA ATCCTGCCTC TTCAACCAAT
601 CCTAACGCCA CGAATGGCGG CAGCGATTTT GGAAGGATAA ATGTAGCTAA
651 TGGCATCAAG CTTGACAGCG GTTCGGAAAA TGTAACGTTG ACACATTGTA
701 AAGACAAAGT ATGCGATAGA GATTCTTAG ATGAAGAAGC ACCACCAAAA
751 TCAGAAATTT AAAAATTAAG TGATGAAGAA AAAATTAATA AATATAAAAA
801 AGACGAGCAA CGAGAGAATT TTGTCGGTTT GGTTGCTGAC AGGGTAGAAA

```


a287.pep

m287/a287 ORFs 287 and 287.a showed a 77.2% identity in 501 aa overlap

	10	20	30	40	50	60
m287.pep	MFKRSVIAMACIFALSACGGGGGGSPDVKSADTL	SKPAAPVVSE-----	KETEA			
a287	MFKRSVIAMACIVAL	SACGGGGGGSPDVKSADTL	SKPAAPVVTTEDV	GEEVLPKEKKDEEA		
	10	20	30	40	50	60
m287.pep	50	60	70	80	90	100
a287	50	60	70	80	90	100
	110	120	130	140	150	160
m287.pep	110	120	130	140	150	160
a287	110	120	130	140	150	160
	170	180	190	200	210	220
m287.pep	170	180	190	200	210	220
a287	170	180	190	200	210	220
	230	240	250	260	270	280
m287.pep	230	240	250	260	270	280
a287	230	240	250	260	270	280
	290	300	310	320	330	340
m287.pep	290	300	310	320	330	340

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```

a287      KSASSSSARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRY
           300      310      320      330      340      350

m287.pep  350      360      370      380      390      400
           LTYGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDF
           ||||| ||||| ||||| |||||:||||| ||||| ||||| |: |||||
a287      LTYGAEKLSGGSYALSVQGEPAKGEMLAGTAVYNGEVLHFHMENGRPSPSGGRFAAKVDF
           360      370      380      390      400      410

m287.pep  410      420      430      440      450      460
           GSKSVVDGIIDSGDDLHMGTKFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYS
           ||||| ||||| ||||| |||||:||||| ||||| |||||:||||| |||||
a287      GSKSVVDGIIDSGDDLHMGTKFKKAVIDGNGFKGTWTENGSGGDVSGRFYGPAGEEVAGKYS
           420      430      440      450      460      470

m287.pep  470      480      489
           YRPTDAEKGGFGVFAGKKEQDX
           ||||| ||||| ||||| |||||
a287      YRPTDAEKGGFGVFAGKKEQDX
           480      490

```

406

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 74>:

```

m406.seq
1  ATGCAAGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG ACGTACGACG GTATTCCATC GCATGGCGGA GGTAAACGCT
101 TTGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTAAAT
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 CACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTCGTGGC GAATACATAA ACAGCCCTGC CGTCCGTACC
301 GATTACACCT ATCCACGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACAGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CTCGACCCCA ATCAGACGGT AGCGGAAGTA AAAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CTAACCCGCG
501 CGACACTGCC TTTCTTTCCC ACTTGGTACA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTTCTCCT GCCAATGCCG ATACAGATGT GTTTATTAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAACT GGAATATTTT GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCAA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGGCCGTATA AAGTAAGCAA
801 AGGAATTAAA CCGACGGAAG GATTAATGGT CGATTTCTCC GATATCCGAC
851 CATACGGCAA TCATACGGGT AACTCCGCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GTAGTGCAGC AACATAGACA
951 AGGACAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 75; ORF 406>:

```

m406.pep
1  MQARLLIPIL FSVFILSACG TLTGIPSHGG GKRFVEQEL VAASARAQVK
51  DMDLQALHGR KVALYIATMG DQSGSGLTGG RYSIDALIRG EYINSPAVRT
101 DYTPRYETT AETTSGLTGT LTTSLSTLNA PALSRQSDG SGSKSSLGLN
151 IGGMGDYRNE TLTTNPRDTA FLSHLVQTVF FLRGIDVVSF ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSKEGIK PTEGLMVDFFS DIRPYGNHTG NSAPSVEADN
301 SHEGYGSDE VVRQHRQGPQ *

```

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The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 76>:

g406.seq

```

1  ATGCGGGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG ACACTGACAG GTATTCCATC GCATGGCGGA GGCAAACGCT
101 TCGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 AACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTTCGCGC GAATACATAA ACAGCCCTGC CGTCCGCACC
301 GATTACACCT ATCCGCGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACGGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CGCGCACCCA ATCAGACGGT AGCGGAAGTA GGAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CCAACCCGCG
501 CGACACTGCC TTTCTTTCCC ACTTGGTGCA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTTCTCCT GCCAATGCCG ATACAGATGT GTTTATTAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAAAC TGAATATTTT GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCCA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGGCCGTATA AAGTAAGCAA
801 AGGAATCAAA CCGACGGAAG GATTGATGGT CGATTCTCTC GATATCCAAC
851 CATACGGCAA TCATACGGGT AACTCCGCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GCAGTGCAC AACATAGACA
951 AGGGCAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 77; ORF 406.ng>:

g406.pep

```

1  MRARLLIPIL FSVFILSACG TLTGIPSHGG GKRFVEQEL VAASARA AVK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTPRYETT AETTSGLTG LTTSLSTLNA PALSRQSDG SGSRSSLGLN
151 IGGMGDYRNE TLTTNPRDTA FLSHLVQTVF FLRGIDVVS ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSKEIK PTEGLMVD FS DIQPYGNHTG NSAPSVEADN
301 SHEGYGSDE AVRQHRQGP *

```

ORF 406.ng shows 98.8% identity over a 320 aa overlap with a predicted ORF (ORF406.a) from *N. gonorrhoeae*:

g406/m406

	10	20	30	40	50	60
g406.pep	MRARLLIPILFSVFILSACGLTLTGIPSHGGGKRFVEQELVAASARA AVKDMDLQALHGR					
m406	MQARLLIPILFSVFILSACGLTLTGIPSHGGGKRFVEQELVAASARA AVKDMDLQALHGR					
	10	20	30	40	50	60
g406.pep	KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTG					
m406	KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTG					
	70	80	90	100	110	120
g406.pep	LTTSLSTLNAPALSRQSDGSGSRSSLGLNIGGMGDYRNETLTTNPRDTAFLSHLVQTVF					
m406	LTTSLSTLNAPALSRQSDGSGSKSSLGLNIGGMGDYRNETLTTNPRDTAFLSHLVQTVF					
	130	140	150	160	170	180
g406.pep	LTTSLSTLNAPALSRQSDGSGSKSSLGLNIGGMGDYRNETLTTNPRDTAFLSHLVQTVF					
m406	LTTSLSTLNAPALSRQSDGSGSKSSLGLNIGGMGDYRNETLTTNPRDTAFLSHLVQTVF					
	190	200	210	220	230	240

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```

g406.pep  FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||
m406      FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
          190      200      210      220      230      240

          250      260      270      280      290      300
g406.pep  IKPKTNAFEAAYKENYALWMGPYKVSCKIKPTEGLMVDFSIDIQPYGNHTGNSAPSVEADN
          ||||||||||||||||||||||||||||||||||||||||:|||||||||||||
m406      IKPKTNAFEAAYKENYALWMGPYKVSCKIKPTEGLMVDFSIDIRPYGNHTGNSAPSVEADN
          250      260      270      280      290      300

          310      320
g406.pep  SHEGYGYSDEAVRQHRQGPX
          |||||||||:|||||||
m406      SHEGYGYSDEVVRQHRQGPX
          310      320

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 78>:

```

a406.seq
1   ATGCAAGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG AACTGACAG GTATTCCATC GCATGGCGGA GGTAACGCT
101 TCGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 AACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTCGTGGC GAATACATAA ACAGCCCTGC CGTCCGTACC
301 GATTACACCT ATCCACGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACAGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CGCGCACCCA ATCAGACGGT AGCGGAAGTA AAAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CTAACCCGCG
501 CGACACTGCC TTTCTTTCCC ACTTGGTACA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTCCTCCT GCCAATGCCG ATACGGATGT GTTTATTAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAACT GGAATATTTC GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCAA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGACCGTATA AAGTAAGCAA
801 AGGAATTAAA CCGACAGAAG GATTAATGGT CGATTTCTCC GATATCCAAC
851 CATACGGCAA TCATATGGGT AACTCTGCCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GCAGTGCAGC GACATAGACA
951 AGGGCAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 79; ORF 406.a>:

```

a406.pep
1   MQARLLIPIL FSVFILSACG TLTGIPSHGG GKRFQVEQEL VAASARAQVK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTYPYRSETT AETTSGLTGT LTSLSTLNA PALSRTQSDG SGSKSSLGLN
151 IGGMGDYRNE TLTTNPRDTA FLHLVQTVF FLRGIDVVSP ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRNTNKKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSCKIK PTEGLMVDFS DIQPYGNHMG NSAPSVEADN
301 SHEGYGYSDE AVRRHRQGPX *

```

m406/a406 ORFs 406 and 406.a showed a 98.8% identity in 320 aa overlap

```

          10      20      30      40      50      60
m406.pep  MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFQVEQELVAASARAQVKDMDLQALHGR
          |||||||||||||||||||||||||||||||||||||||||||||||||||
a406      MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFQVEQELVAASARAQVKDMDLQALHGR
          10      20      30      40      50      60

          70      80      90      100     110     120
m406.pep  KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTYPYRSETTAE
          |||||||||||||||||||||||||||||||||||||||||||||||||||

```

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a406	KVALYIATMGDQGSGLTGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGGLTG
	70 80 90 100 110 120
m406.pep	LTTSLSLTLNAPALSRTQSDGSGSKSSLGLNIGGMGDYRNETLTNNPRDTAFLSHLVQTVF
	130 140 150 160 170 180
a406	LTTSLSLTLNAPALSRTQSDGSGSKSSLGLNIGGMGDYRNETLTNNPRDTAFLSHLVQTVF
	130 140 150 160 170 180
m406.pep	FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
	190 200 210 220 230 240
a406	FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
	190 200 210 220 230 240
m406.pep	IKPKTNAFEAAAYKENYALWMPYKVS KGIKPTEGLMVDFSDIRPYGNHTGNSAPSVEADN
	250 260 270 280 290 300
a406	IKPKTNAFEAAAYKENYALWMPYKVS KGIKPTEGLMVDFSDIQPYGNHMGNSAPSVEADN
	250 260 270 280 290 300
m406.pep	SHEGYGYSDEVVRQHRQGQPX
	310 320
a406	SHEGYGYSDEAVRRHRQGQPX
	310 320

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 80>:

m726.seq

1	ATGACCATCT	ATTTCAAAAA	CGGCTTTTAC	GACGACACAT	TGGGCGGCAT
51	CCCCGAAGGC	GCGGTTGCCG	TCCGCGCCGA	AGAATACGCC	GCCCTTTTGG
101	CAGGACAGGC	GCAGGCGGGG	CAGATTGCCG	CAGATTCCGA	CGGCCGCCCC
151	GTTTTAACCC	CGCCGCGCCC	GTCCGATTAC	CACGAATGGG	ACGGCAAAAA
201	ATGGAATATC	AGCAAAGCCG	CCGCCGCCGC	CCGTTTCGCC	AAACAAAAAA
251	CCGCCTTGCC	ATTCGCGCTC	GCGGAAAAGG	CGGACGAACT	CAAAAACAGC
301	CTCTTGCGG	GCTATCCCCA	AGTGGAATC	GACAGCTTTT	ACAGGCAGGA
351	AAAAGAAGCC	CTCGCGCGGC	AGGCGGACAA	CAACGCCCCG	ACCCCGATGC
401	TGGCGCAAAT	CGCCGCCGCA	AGGGCGGTGG	AATTGGACGT	TTTGATTGAA
451	AAAGTTATCG	AAAAATCCGC	CCGCCTGGCT	GTTGCCGCCG	GCGCGATTAT
501	CGGAAAGCGT	CAGCAGCTCG	AAGACAAATT	GAACACCATC	GAAACCGCGC
551	CCGATTGGA	CGCGCTGGAA	AAGGAAATCG	AAGAATGGAC	GCTAAACATC
601	GGCTGA				

This corresponds to the amino acid sequence <SEQ ID 81; ORF 726>:

m726.pep

1	MTIYFKNGFY	DDTLGGIPEG	AVAVRAEEYA	ALLAGQAQGG	QIAADSDGRP
51	VLTPPRPSDY	HEWDGKKWKI	SKAAAAARFA	KQKTALAFRL	AEKADELKNS
101	LLAGYPQVEI	DSFYRQEKEA	LARQADNNAP	TPMLAQIAAA	RGVELDLVIE
151	KVIEKSARLA	VAAGAIIGKR	QQLEDKLNTI	ETAPGLDALE	KEIEEWTLNI
201	G*				

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 82>:

m907-2.seq

1	ATGAGAAAAAC	CGACCGATAC	CCTACCCGTT	AATCTGCAAC	GCCGCCGCCT
51	GTTGTGTGCC	GCCGCTGCGT	TGTTGCTCAG	TCCTCTGGCG	CACGCCGGCG
101	CGCAACGTGA	GGAAACGCTT	GCCGACGATG	TGGCTTCCGT	GATGAGGAGT

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```

151 TCTGTCGGCA GCGTCAATCC GCCGAGGCTG GTGTTTGACA ATCCGAAAGA
201 GGGCGAGCGT TGGTTGCTG CCATGTCGGC ACGTTTGCCA AGGTTCGTCC
251 CCGAGGAGGA GGAGCGGCGC AGGCTGCTGG TCAATATCCA GTACGAAAGC
301 AGCCGGGCCG GTTTGGATAC GCAGATTGTG TTGGGGCTGA TTGAGGTGGA
351 AAGCGCGTTC CGCCAGTATG CAATCAGCGG TGTCGGCGCG CGCGGCCTGA
401 TGCAGGTTAT GCCGTTTGG AAAAATACTACA TCGGCAAACC GGCGCACAAAC
451 CTGTTCGACA TCCGCACCAA CCTGCGTTAC GGCTGTACCA TCCTGCGCCA
501 TTACCGGAAT CTTGAAAAAG GCAACATCGT CCGCGCGCTT GCCCGCTTTA
551 ACGGCAGCTT GGGCAGCAAT AAATATCCGA ACGCCGTTTT GGGCGCGTGG
601 CGCAACCGCT GGCAGTGGCG TTGA

```

This corresponds to the amino acid sequence <SEQ ID 83; ORF 907-2>:

m907-2.pep

```

1 MRKPTDTLPV NLQRRRLCA AGALLLSPLA HAGAQREETL ADDVASVMRS
51 SVGSVNPPRL VFDNPKGER WLSAMSARLA RFVPEEEERR RLLVNIQYES
101 SRAGLDTQIV LGLIEVESAF RQYAISGVA RGLMQVMPFW KNYIGKPAHN
151 LFDIRTNLRY GCTILRHYRN LEKGNIVRAL ARFNGLSLGSN KYPNAVLAGAW
201 RNRWQWR*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 84>:

m953.seq

```

1 ATGAAAAAAA TCATCTTCGC CGCACTCGCA GCCGCCGCCA TCAGTACTGC
51 CTCCGCCGCC ACCTACAAAG TGGACGAATA TCACGCCAAC GCCCGTTTCG
101 CCATCGACCA TTTCACACC AGCACCAACG TCGGCGGTTT TTACGGTCTG
151 ACCGGTTCCG TCGAGTTCGA CCAAGCAAAA CGCGACGGTA AAATCGACAT
201 AACCATCCCC ATTGCCAACC TGCAAAGCGG TTCGCAACAC TTTACCGACC
251 ACCTGAAATC AGCCGACATC TTCGATGCCG CCCAATATCC GGACATCCGC
301 TTTGTTTCCA CCAAATTCAT CTTCAACGGC AAAAACTGG TTTCCGTTGA
351 CGGCAACCTG ACCATGCACG GCAAACCGC CCCCCTCAA CTCAAAGCCG
401 AAAAATTCAT CTGCTACCAA AGCCCGATGG AGAAAACCGA AGTTTGTGGC
451 GCGGACTTCA GCACCAACCA CGACCGCACC AAATGGGGCA TGGACTACCT
501 CGTTAACGTT GGTATGACCA AAAGCGTCCG CATCGACATC CAAATCGAGG
551 CAGCCAAACA ATAA

```

This corresponds to the amino acid sequence <SEQ ID 85; ORF 953>:

m953.pep

```

1 MKKIIFAALA AAAISTASAA TYKVDEYHAN ARFAIDHFNT STNVGGFYGL
51 TGSVEFDQAK RDGKIDITIP IANLQSGSQH FTDHLKSADI FDAAQYPDIR
101 FVSTKFNENG KKLVSVDGNL TMHGKTAPVK LKAKEFNQYQ SPMEKTEVCG
151 GDFSTTIDRT KWGMDYLVNV GMTKSVRIDI QIEAAKQ*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 86>:

orf1-1.seq

```

1 ATGAAAACAA CCGACAAACG GACAACCGAA ACACACCGCA AAGCCCCGAA
51 AACCGGCCGC ATCCGCTTCT CGCCTGCTTA CTTAGCCATA TGCCTGTCGT
101 TCGGCATTCT TCCCCAAGCC TGGGCGGGAC ACACTTATTT CGGCATCAAC
151 TACCAATACT ATCGCGACTT TGCCGAAAAT AAAGGCAAGT TTGCAGTCGG
201 GGCGAAAGAT ATTGAGGTTT ACAACAAAAA AGGGGAGTTG GTCGGCAAT
251 CAATGACAAA AGCCCCGATG ATTGATTTTT CTGTGGTGTC GCGTAACGGC
301 GTGGCGGCAT TGGTGGGCGA TCAATATATT GTGAGCGTGG CACATAACGG
351 CGGCTATAAC AACGTTGATT TTGGTGCGGA AGGAAGAAAT CCCGATCAAC
401 ATCGTTTTAC TTATAAAATT GTGAAACGGA ATAATTATAA AGCAGGGACT
451 AAAGGCCATC CTTATGGCGG CGATTATCAT ATGCCGCGTT TGCATAAATT
501 TGTCACAGAT GCAGAACCTG TTGAAATGAC CAGTTATATG GATGGGCGGA

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551 AATATATCGA TCAAAATAAT TACCCTGACC GTGTTCGTAT TGGGGCAGGC
 601 AGGCAATATT GGCGATCTGA TGAAGATGAG CCCAATAACC GCGAAAGTTC
 651 ATATCATATT GCAAGTGCGT ATTCTTGGCT CGTTGGTGGC AATACCTTTG
 701 CACAAAATGG ATCAGGTGGT GGCACAGTCA ACTTAGGTAG TGAAAAAATT
 751 AAACATAGCC CATATGGTTT TTTACCAACA GGAGGCTCAT TTGGCGACAG
 801 TGGCTCACCA ATGTTTATCT ATGATGCCCA AAAGCAAAAG TGGTTAATTA
 851 ATGGGGTATT GCAAACGGGC AACCCTTATA TAGGAAAAAG CAATGGCTTC
 901 CAGCTGGTTC GTAAAGATTG GTTCTATGAT GAAATCTTTG CTGGAGATAC
 951 CCATTTCAGTA TTCTACGAAC CACGTCAAAA TGGGAAATAC TCTTTTAACG
 1001 ACGATAATAA TGGCACAGGA AAAATCAATG CCAAACATGA ACACAATTCT
 1051 CTGCCTAATA GATTAAAAAC ACGAACCGTT CAATTGTTTA ATGTTTCTTT
 1101 ATCCGAGACA GCAAGAGAAC CTGTTTATCA TGCTGCAGGT GGTGTCAACA
 1151 GTTATCGACC CAGACTGAAT AATGGAGAAA ATATTTCTTT TATTGACGAA
 1201 GGAAAAGGCG AATTGATACT TACCAGCAAC ATCAATCAAG GTGCTGGAGG
 1251 ATTATATTTT CAAGGAGATT TTACGGTCTC GCCTGAAAAT AACGAACTT
 1301 GGCAAGGCGC GGGCGTTCAT ATCAGTGAAG ACAGTACCGT TACTTGGAAA
 1351 GTAAACGGCG TGGCAAACGA CCGCCTGTCC AAAATCGGCA AAGGCACGCT
 1401 GCACGTTCAA GCCAAAGGGG AAAACCAAGG CTCGATCAGC GTGGGCGACG
 1451 GTACAGTCAT TTTGGATCAG CAGGCAGACG ATAAAGGCAA AAAACAAGCC
 1501 TTTAGTGAAA TCGGCTTGGT CAGCGGCAGG GGTACGGTGC AACTGAATGC
 1551 CGATAATCAG TTCAACCCCG ACAACTCTA TTTCCGGCTT CGCGGCGGAC
 1601 GTTTGGATTT AAACGGGCAT TCGCTTTCGT TCCACCGTAT TCAAAATACC
 1651 GATGAAGGGG CGATGATTGT CAACCACAAT CAAGACAAAG AATCCACCGT
 1701 TACCATTACA GGCAATAAAG ATATTGCTAC AACC GGCAAT AACAACAGCT
 1751 TGGATAGCAA AAAAGAAATT GCCTACAACG GTTGGTTTGG CGAGAAAGAT
 1801 ACGACCAAAA CGAACGGGCG GCTCAACCTT GTTTACCAGC CCGCCGAGCA
 1851 AGACCGCACC CTGCTGCTTT CCGGCGGAAC AAATTTAAAC GGCAACATCA
 1901 CGCAAACAAA CGGCAAACTG TTTTTCAGCG GCAGACCAAC ACCGACGCC
 1951 TACAATCATT TAAACGACCA TTGGTCGCAA AAAGAGGGCA TTCCTCGCGG
 2001 GGAAATCGTG TGGGACAACG ACTGGATCAA CCGCACATTT AAAGCGGAAA
 2051 ACTTCCAAAT TAAAGCGGGA CAGGCGGTGG TTTCCCGCAA TGTTGCCAAA
 2101 GTGAAAGGCG ATTGGCATTG GAGCAATCAC GCCCAAGCAG TTTTGGTGT
 2151 CGCACCGCAT CAAAGCCACA CAATCTGTAC ACGTTCGGAC TGGACGGTTC
 2201 TGACAAATTG TGTCGAAAAA ACCATTACCG ACGATAAAGT GATTGCTTCA
 2251 TTGACTAAGA CCGACATCAG CGGCAATGTC GATCTTGCCG ATCAGCTCA
 2301 TTAAATCTC ACAGGGCTTG CCACACTCAA CGGCAATCTT AGTGCAAATG
 2351 GCGATACACG TTATACAGTC AGCCACAACG CCACCCAAAA CGGCAACCTT
 2401 AGCTCGTGG GCAATGCCCA AGCAACATTT AATCAAGCCA CATTAAACGG
 2451 CAACACATCG GCTTCGGGCA ATGCTTCATT TAATCTAAGC GACCACGCCG
 2501 TACAAAACGG CAGTCTGACG CTTTCCGGCA ACGCTAAGGC AAACGTAAGC
 2551 CATTCCGCAC TCAACGGTAA TGTCTCCCTA GCCGATAAGG CAGTATTCCA
 2601 TTTTGAAAGC AGCCGCTTTA CCGGACAAAT CAGCGGCGGC AAGGATACGG
 2651 CATTACACTT AAAAGACAGC GAATGGACGC TGCCGTCAGG CACGGAATTA
 2701 GGCAATTTAA ACCTTGACAA CGCCACCATT ACACTCAATT CCGCCTATCG
 2751 CCACGATGCG GCAGGGGCGC AAACCGGCAG TCGCAGAGAT GCGCCGCGCC
 2801 GCCGTTCCGC CCGTTCGCGC CGTTCCCTAT TATCCGTTAC ACCGCCAACT
 2851 TCGGTAGAAT CCCGTTTCAA CACGCTGACG GTAAACGGCA AATTGAACGG
 2901 TCAGGGAACA TTCCGCTTTA TGTCGGAAC TTTCCGCTAC CGCAGCGACA
 2951 AATTGAAGCT GGCGGAAAGT TCCGAAGGCA CTTACACCTT GGCGGTCAAC
 3001 AATACCGGCA ACGAACCTGC AAGCCTCGAA CAATTGACGG TAGTGGAAGG
 3051 AAAAGACAAC AAACCGCTGT CCGAAAACCT TAATTTCAAC CTGCAAAACG
 3101 AACACGTCGA TGCCGGCGCG TGCGCTTACC AACTCATCCG CAAAGACGGC
 3151 GAGTTCCGCC TGCATAATCC GGTCAAAGAA CAAGAGCTTT CCGACAAACT
 3201 CGGCAAGGCA GAAGCCAAAA AACAGGCGGA AAAAGACAAC GCGCAAAGCC
 3251 TTGACGCGCT GATTGCGGCC GGGCGCGATG CCGTCGAAAA GACAGAAAGC
 3301 GTTGCCGAAC CGGCCCGGCA GGCAGGCGGG GAAAATGTCT GCATTATGCA
 3351 GGCGGAGGAA GAGAAAAAAC GGGTGCAGGC GGATAAAGAC ACCGCCTTGG
 3401 CGAAACAGCG CGAAGCGGAA ACCCGGCCGG CTACCACCGC CTTCCCTCCG
 3451 GCGGCGCGCG CCGGCCGGGA TTTGCCGCAA CTGCAACCCC AACCAGAGCC
 3501 CCAACCGCAG CGCGACCTGA TCAGCCGTTA TGCCAATAGC GGTGTTGAGT
 3551 AATTTTCCGC CACGCTCAAC AGCGTTTTCG CCGTACAGGA CGAATTAGAC
 3601 CGCGTATTTG CCGAAGACCG CCGCAACGCC GTTTGGACAA GCGGCATCCG
 3651 GGACACCAAA CACTACCGTT CGCAAGATTT CCGCGCCTAC CGCCAACAAA

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3701 CCGACCTGCG CCAATCGGT ATGCAGAAAA ACCTCGGCAG CGGGCGCGTC
3751 GGCATCCTGT TTTCGCACAA CCGGACCGAA AACACCTTCG ACGACGGCAT
3801 CGGCAACTCG GCACGGCTTG CCCACGGCGC CGTTTTTCGG CAATACGGCA
3851 TCGACAGGTT CTACATCGGC ATCAGCGCGG GCGCGGGTTT TAGCAGCGGC
3901 AGCCTTTCAG ACGGCATCGG AGGCAAAATC CGCCGCCGCG TGCTGCATTA
3951 CGGCATTTCAG GCACGATACC GCGCCGGTTT CGGCGGATTC GGCATCGAAC
4001 CGCACATCGG CGCAACGCGC TATTTCTGCC AAAAAGCGGA TTACCGCTAC
4051 GAAAACGTCA ATATCGCCAC CCCCAGCCTT GCATTCAACC GCTACCGCGC
4101 GGGCATTAAG GCAGATTATT CATTCAAACC GGCGCAACAC ATTTCCATCA
4151 CGCCTTATTT GAGCCTGTCC TATACCGATG CCGCTTCGGG CAAAGTCCGA
4201 ACACGCGTCA ATACCGCGGT ATTGGCTCAG GATTTTCGGCA AAACCCGCAG
4251 TGCGGAATGG GGCGTAAACG CCGAAATCAA AGGTTTCACG CTGTCCCTCC
4301 ACGCTGCCGC CGCCAAAGGC CCGCAACTGG AAGCGCAACA CAGCGCGGGC
4351 ATCAAATTAG GCTACCGCTG GTAA

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This corresponds to the amino acid sequence <SEQ ID 87; ORF orf1-1>:

orf1-1.pep

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1 MKTTDKRTTE THRKAPKTGR IRFSPAYLAI CLSFGILPQA WAGHTYFGIN
51 YQYYRDFEEN KGKFAVGAKD IEVYNKKGEL VGKSMTKAPM IDFSVVSRRG
101 VAALVGDQYI VSAHNGGYN NVDFGAERGN PDQHRFTYKI VKRNNYKAGT
151 KGHYPGGDYH MPRLHKFVTD AEPVEMTSYM DGRKYIDQNN YPDRVRIGAG
201 RQYWRSDDEE PNNRESSYHI ASAYSWLVGG NTFAQNGSGG GTVNLGSEKI
251 KHSPYGFLLPT GGSFGDSGSP MFIYDAQKQK WLINGVLQTG NPYIGKSNFG
301 QLVKRDWFYD EIFAGDTHSV FYEPRQNGKY SFNDDNNGTG KINAKHEHNS
351 LPNRLKTRTV QLFNVSLSET AREPVYHAAG GVNSYRPRLN NGENISFIDE
401 KGELILTSN INQAGGLYF QGDFTVSPEN NETWQAGGVH ISEDSTVTWK
451 VNGVANDRLS KIGKGTLHVQ AKGENQGSIS VGDGTVILDQ QADDKGGKQA
501 FSEIGLVSGR GTVQLNADNQ FNPDKLYFGF RGGRLDLNGH SLSFHRIQNT
551 DEGAMIVNHN QDKESTVTIT GNKDIATGN NNSLDSKKEI AYNGWFGEKD
601 TTKTNGRLNL VYQPAEDRT LLLSGGTNLN GNITQTNGKL FFSGRPTPHA
651 YNHLNDHWSQ KEGIPRGEIV WDNDWINRTF KAENFQIKGG QAVVSRNVAK
701 VKGDWHLNSH AQAVFGVAPH QSHTICTRSD WTGLTNCVEK TITDDKVIAS
751 LTKTDISGNV DLADHAHLNL TGLATLNGNL SANGDTRYTV SHNATQNGNL
801 SLVGNAQATF NQATLNGNTS ASGNASFNLS DHAVQNGSLT LSGNAKANVS
851 HSAIANGVSL ADKAVFHFES SRFTGQISGG KDTALHLKDS EWTLPSTGTE
901 GNILNDNATI TLNSAYRHDA AGAQTGSATD APRRRSRRSR RSLLSVTPPT
951 SVESRENTLT VNGKLNQGT FRFMSLEFGY RSDKLKLAES SEGTYTLAVN
1001 NTGNEPASLE QLTVEGKDN KPLSENLFNT LQNEHVDAGA WRYQLIRKDG
1051 EFRILHNPVKE QELSDKLKGA EAKKQAEKDN AQSLDALIAA GRDAVEKTES
1101 VAEPARQAGG ENVGIMQAE EKKRVQADKD TALAKQREAE TRPATTAFFR
1151 ARRARRDLPO LQPQPQPQPO RDLISRYANS GLSEFSATLN SVFAVQDELD
1201 RVFAEDRRNA VWTSGIRDTK HYRSQDFRAY RQOTDLRQIG MQKNLGSGRV
1251 GILFSHNRT E NTFDDGIGNS ARLAHGAVFG QYGIDRFYIG ISAGAGFSSG
1301 SLSDGIGGKI RRRVLHYGIQ ARYRAGFGGF GIEPHIGATR YFVQKADYRY
1351 ENVNIATPGL AFNRYRAGIK ADYSFKPAQH ISITPYLSLS YTDAAAGKVR
1401 TRVNTAVLAQ DFGKTRSAEW GVNAEIKGFT LSLHAAAAGK PQLEAQHSAG
1451 IKLYRW*

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The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 88>:

orf46-2.seq

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1 TTGGGCATTT CCCGCAAAAT ATCCCTTATT CTGTCCATAC TGGCAGTGTG
51 CCTGCCGATG CATGCACACG CCTCAGATTT GGCAAACGAT TCTTTTATCC
101 GGCAGGTTCT CGACCGTCAG CATTTCGAAC CCGACGGGAA ATACCACCTA
151 TTCGCAGCA GGGGGGAACT TGCCGAGCGC AGCGGCCATA TCGGATTGGG
201 AAAAATACAA AGCCATCAGT TGGGCAACCT GATGATTCAA CAGCGGCCCA
251 TTAAAGGAAA TATCGGCTAC ATTGTCGCTT TTTCCGATCA CGGGCACGAA
301 GTCCATTCCC CCTTCGACAA CCATGCCTCA CATTCCGATT CTGATGAAGC
351 CCGTAGTCCC GTTGACGGAT TTAGCCTTTA CCGCATCCAT TGGGACGGAT

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401  ACGAACACCA TCCCGCCGAC GGCTATGACG GGCCACAGGG CGGCGGCTAT
451  CCCGCTCCCA AAGGCGCGAG GGATATATAC AGCTACGACA TAAAAGGCGT
501  TGCCCAAAAT ATCCGCCTCA ACCTGACCGA CAACCGCAGC ACCGGACAAC
551  GGCTTGCCGA CCGTTTCCAC AATGCCGGTA GTATGCTGAC GCAAGGAGTA
601  GGCGACGGAT TCAAACGCGC CACCCGATAC AGCCCCGAGC TGGACAGATC
651  GGGCAATGCC GCCGAAGCCT TCAACGGCAC TGCAGATATC GTTAAAAACA
701  TCATCGGCGC GGCAGGAGAA ATTGTCGGCG CAGGCGATGC CGTGCAGGGC
751  ATAAGCGAAG GCTCAAACAT TGCTGTCATG CACGGCTTGG GTCTGCTTTC
801  CACCGAAAAAC AAGATGGCGC GCATCAACGA TTTGGCAGAT ATGGCGCAAC
851  TCAAAGACTA TGCCGCAGCA GCCATCCGCG ATTGGGCAGT CCAAAACCCC
901  AATGCCGCAC AAGGCATAGA AGCCGTCAGC AATATCTTTA TGGCAGCCAT
951  CCCCATCAAA GGGATTGGAG CTGTTCCGGG AAAATACGGC TTGGGCGGCA
1001 TCACGGCACA TCCTATCAAG CGGTCGCAGA TGGGCGCGAT CGCATTGCCG
1051 AAAGGGAAAT CCGCCGTCAG CGACAATTTT GCCGATGCGG CATACGCCAA
1101 ATACCCGTCC CTTTACCATT CCCGAAATAT CCGTTCAAAC TTGGAGCAGC
1151 GTTACGGCAA AGAAAACATC ACCTCCTCAA CCGTGCCGCC GTCAAACGGC
1201 AAAAATGTCA AACTGGCAGA CCAACGCCAC CCGAAGACAG GCGTACCGTT
1251 TGACGGTAAA GGGTTTCCGA ATTTTGAGAA GCACGTGAAA TATGATACGA
1301 AGCTCGATAT TCAAGAATTA TCGGGGGGCG GTATACCTAA GGCTAAGCCT
1351 GTGTTTGATG CGAAACCGAG ATGGGAGGTT GATAGGAAGC TTAATAAATT
1401 GACAACTCGT GAGCAGGTGG AGAAAAATGT TCAGGAAATA AGGAACGGTA
1451 ATATAAACAG TAACTTTAGC CAACATGCTC AACTAGAGAG GGAAATTAAT
1501 AACTAAAAAT CTGCCGATGA AATTAATTTT GCAGATGGAA TGGGAAAATT
1551 TACCGATAGC ATGAATGACA AGGCTTTTAG TAGGCTTGTG AAATCAKTTA
1601 AAGAGAATGG CTTACAAAAT CCAGTTGTGG AGTACGTTGA AATAAATGGA
1651 AAAGCATATA TCGTAAGAGG AAATAATRGG GTTTTTGCTG CAGAATACCT
1701 TGGCAGGATA CATGAATTAA AATTTAAAAA AGTTGACTTT CCTGTTCCCTA
1751 ATACTAGTTG GAAAAATCCT ACTGATGTCT TGAATGAATC AGGTAATGTT
1801 AAGAGACCTC GTTATAGGAG TAAATAA

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This corresponds to the amino acid sequence <SEQ ID 89; ORF orf46-2>:

orf46-2.pep

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1  LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRO HFEPDGKYHL
51  FGSRGELAER SGHIGLGKIQ SHQLGNLMIQ QAAIKGNIGY IVRFSDDHGE
101 VHSFPDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHHPAD GYDGPQGGGY
151 PAPKGARDIY SYDIKVAQN IRLNLTDNRS TGQRLADRFH NAGSMLTQGV
201 GDGFKRATRY SPELDRSGNA AEAFTGTADI VKNIIGAAGE IVGAGDAVQG
251 ISEGSNIAVM HGLGLLSTEN KMARINDLAD MAQLKDYAAA AIRDWAVQNP
301 NAAQGIEAVS NIFMAAIPK GIGAVRGKYG LGGITAHPIK RSQMGAIALP
351 KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI TSSTVPPSNG
401 KNVKLADQRH PKTGVPFDGK GFPNFEKHVK YDTKLDIQEL SGGGIPKAKP
451 VFDAKPRWEV DRKLNKLTR EQVEKNVQEI RNGNINSNFS QHAQLEREIN
501 KLKSADEINF ADGMGKFTDS MNDKAFSRLV KSVKENGFTN PVVEYVEING
551 KAYIVRGNNR VFAAEYLGRI HELKFKKVD FVPNTSWKNP TDVLNESGNV
601 KRPRYRSK*

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Using the above-described procedures, the following oligonucleotide primers were employed in the polymerase chain reaction (PCR) assay in order to clone the ORFs as indicated:

Oligonucleotides used for PCR

Table 1

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ORF	Primer	Sequence	Restriction sites
279	Forward	CGCGGATCCCATATG-TTGCCTGCAATCACGATT <SEQ ID 90>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTAGAAGCGGGCGGCAA <SEQ ID 91>	XhoI
519	Forward	CGCGGATCCCATATG-TTCAAATCCTTTGTCGTCA <SEQ ID 92>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTGGCGGTTTTGCTGC <SEQ ID 93>	XhoI
576	Forward	CGCGGATCCCATATG-GCCGCCCCCGCATCT <SEQ ID 94>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATTTACTTTTTTGATGTCGAC <SEQ ID 95>	XhoI
919	Forward	CGCGGATCCCATATG-TGCCAAAGCAAGAGCATC <SEQ ID 96>	BamHI-NdeI
	Reverse	CCCGCTCGAG-CGGGCGGTATTCGGG <SEQ ID 97>	XhoI
121	Forward	CGCGGATCCCATATG-GAAACACAGCTTTACAT <SEQ ID 98>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATAATAATATCCCGCGCCC <SEQ ID 99>	XhoI
128	Forward	CGCGGATCCCATATG-ACTGACAACGCACT <SEQ ID 100>	BamHI-NdeI
	Reverse	CCCGCTCGAG-GACCGCGTTGTCGAAA <SEQ ID 101>	XhoI
206	Forward	CGCGGATCCCATATG-AAACACCGCCAACCGA <SEQ ID 102>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTCTGTAAAAAAGTATGTGC <SEQ ID 103>	XhoI
287	Forward	CCGGAATTCTAGCTAGC-CTTTCAGCCTGCGGG <SEQ ID 104>	EcoRI-NheI
	Reverse	CCCGCTCGAG-ATCCTGCTCTTTTTTGCC <SEQ ID 105>	XhoI
406	Forward	CGCGGATCCCATATG-TGCGGGACACTGACAG <SEQ ID 106>	BamHI-NdeI
	Reverse	CCCGCTCGAG-AGGTTGTCCTTGTCTATG <SEQ ID 107>	XhoI

EXAMPLE 2

Expression of ORF 919

The primer described in Table 1 for ORF 919 was used to locate and clone ORF 919. The predicted gene 919 was cloned in pET vector and expressed in *E. coli*. The product of

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protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 919-His fusion protein purification. Mice were immunized with the purified 919-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; PP, purified protein, TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 919 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 919 are provided in Figure 10. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 919 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 3

Expression of ORF 279

The primer described in Table 1 for ORF 279 was used to locate and clone ORF 279. The predicted gene 279 was cloned in pGex vector and expressed in *E. coli*. The product of protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 279-GST purification. Mice were immunized with the purified 279-GST and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 279 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 279 are provided in Figure 11. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 279 and the amino acid sequence encoded thereby is provided in Example 1.

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EXAMPLE 4

Expression of ORF 576

The primer described in Table 1 for ORF 576 was used to locate and clone ORF 576. The predicted gene 576 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 576-GST fusion protein purification. Mice were immunized with the purified 576-GST and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B).. These experiments confirm that ORF 576 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 576 are provided in Figure 12. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 576 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 5

Expression of ORF 519

The primer described in Table 1 for ORF 519 was used to locate and clone ORF 519. The predicted gene 519 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 519-His fusion protein purification. Mice were immunized with the purified 519-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 519 is a surface-exposed protein

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and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 519 are provided in Figure 13. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 519 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 6

Expression of ORF 121

The primer described in Table 1 for ORF 121 was used to locate and clone ORF 121. The predicted gene *121* was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 121-His fusion protein purification. Mice were immunized with the purified 121-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 121 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 121 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 121 are provided in Figure 14. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 121 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 7

Expression of ORF 128

The primer described in Table 1 for ORF 128 was used to locate and clone ORF 128. The predicted gene *128* was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 128-His purification. Mice were immunized with the purified 128-His and sera were used for

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Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D) and ELISA assay (panel E). Results show that 128 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 128 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 128 are provided in Figure 15. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 128 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 8

Expression of ORF 206

The primer described in Table 1 for ORF 206 was used to locate and clone ORF 206. The predicted gene 206 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 206-His purification. Mice were immunized with the purified 206-His and sera were used for Western blot analysis (panel B). It is worthnoting that the immunoreactive band in protein extracts from meningococcus is 38 kDa instead of 17 kDa (panel A). To gain information on the nature of this antibody staining we expressed ORF 206 in *E. coli* without the His-tag and including the predicted leader peptide. Western blot analysis on total protein extracts from *E. coli* expressing this native form of the 206 protein showed a recative band at a position of 38 kDa, as observed in meningococcus. We conclude that the 38 kDa band in panel B) is specific and that anti-206 antibodies, likely recognize a multimeric protein complex. In panel C is shown the FACS analysis, in panel D the bactericidal assay, and in panel E) the ELISA assay. Results show that 206 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 206 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots,

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antigenic index, and amphipatic regions of ORF 519 are provided in Figure 16. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 206 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 9

Expression of ORF 287

The primer described in Table 1 for ORF 287 was used to locate and clone ORF 287. The predicted gene 287 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 287-GST fusion protein purification. Mice were immunized with the purified 287-GST and sera were used for FACS analysis (panel B), bactericidal assay (panel C), and ELISA assay (panel D). Results show that 287 is a surface-exposed protein. Symbols: M1, molecular weight marker. Arrow indicates the position of the main recombinant protein product (A). These experiments confirm that 287 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 287 are provided in Figure 17. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 287 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 10

Expression of ORF 406

The primer described in Table 1 for ORF 406 was used to locate and clone ORF 406. The predicted gene 406 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 406-His fusion protein purification. Mice were immunized with the purified 406-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 406 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N.*

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meningitidis outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 406 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipathic regions of ORF 406 are provided in Figure 18. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 406 and the amino acid sequence encoded thereby is provided in Example 1.

The foregoing examples are intended to illustrate but not to limit the invention.

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Claims

1. A method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within one or more of *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
2. A method according to claim 1, comprising the steps of searching a *N. meningitidis* nucleotide sequence for an initiation codon and searching the upstream sequence for an in-frame termination codon.
3. A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to any one of claims 1-2.
4. A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 3, producing an antibody which binds to the protein, and determining whether the antibody recognises a protein produced by *N. meningitidis*.
5. Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to any one of claims 1-2.
6. A protein obtained by the method of claim 3.
7. Nucleic acid comprising one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
8. Nucleic acid comprising a nucleotide sequence having greater than 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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9. Nucleic acid comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

10. Nucleic acid according to claim 9, wherein the fragment is unique to the genome of *N. meningitidis*.

11. Nucleic acid complementary to the nucleic acid of any one of claims 7-10.

12. A protein comprising an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

13. A protein comprising an amino acid sequences having greater than 50% sequence identity to an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

14. A protein comprising a fragment of an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

15. Nucleic acid encoding a protein according to any one of claims 6-8.

16. A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to any one of claims 7-11.

17. A computer, a computer memory, a computer storage medium or a computer database containing one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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18. A polyclonal or monoclonal antibody which binds to a protein according to any one of claims 12-14 or 6.

19. A nucleic acid probe comprising nucleic acid according to any one of claims 5, 7-10, or 15.

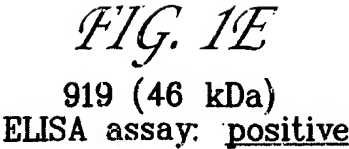
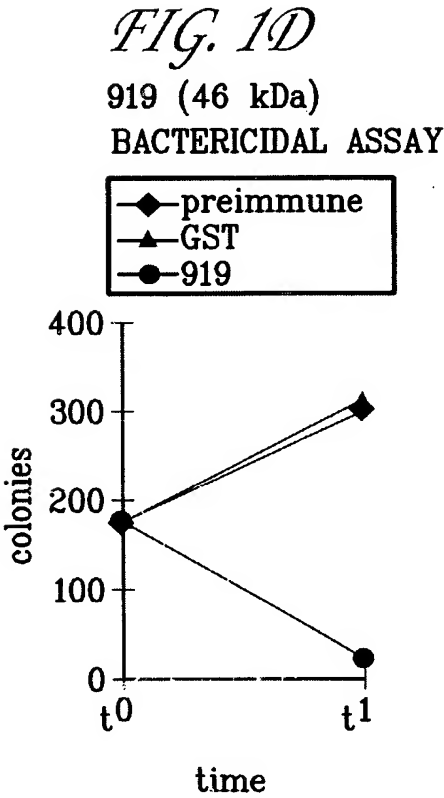
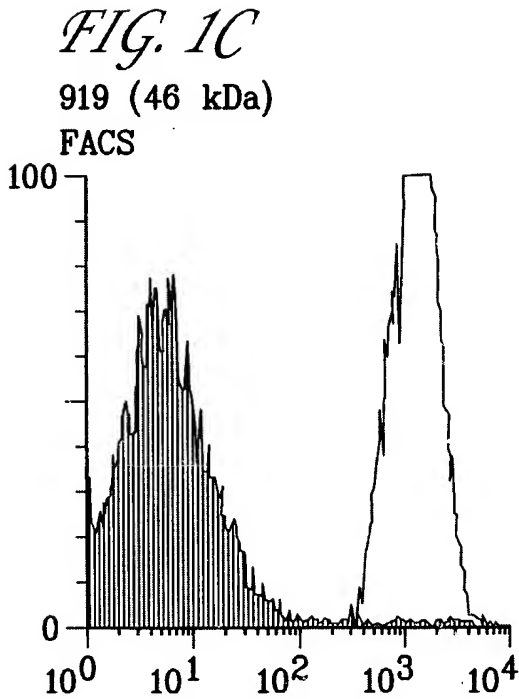
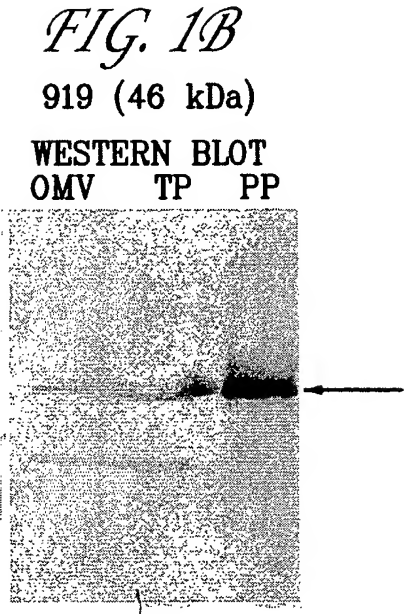
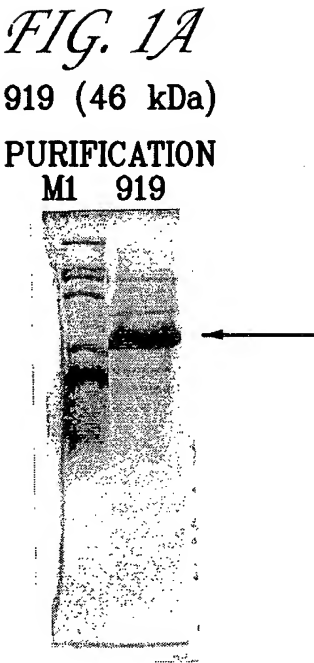
20. An amplification primer comprising nucleic acid according to any one of claims 5, 7-10, or 15.

21. A composition comprising (a) nucleic acid according to any one of claims 5, 7-10, or 15; (b) protein according to any one of claims 12-14; and/or (c) an antibody according to claim 18.

22. The use of a composition according to claim 21 as a medicament or as a diagnostic reagent.

23. The use of a composition according to claim 21 in the manufacture of (a) a medicament for treating or preventing infection due to Neisserial bacteria and/or (b) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria.

24. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 21.



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FIG. 2A

279 (10.5 kDa)
PURIFICATION
M1 279

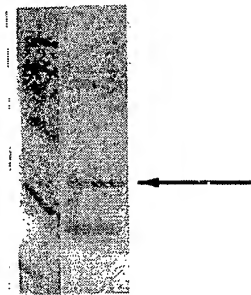


FIG. 2B

279 (10.5 kDa)
WESTERN BLOT
TP OMV

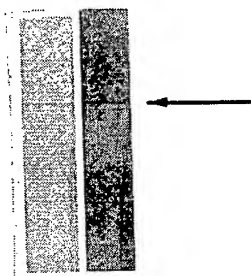


FIG. 2C

279 (10.5 kDa)
FACS

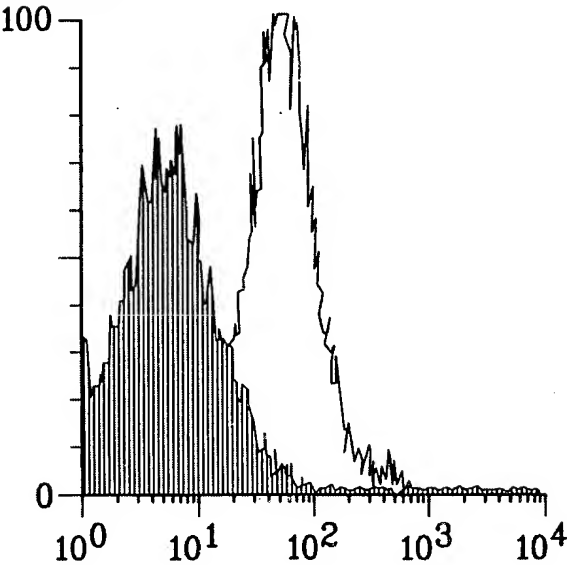


FIG. 2D

279 (10.5 kDa)
BACTERICIDAL ASSAY

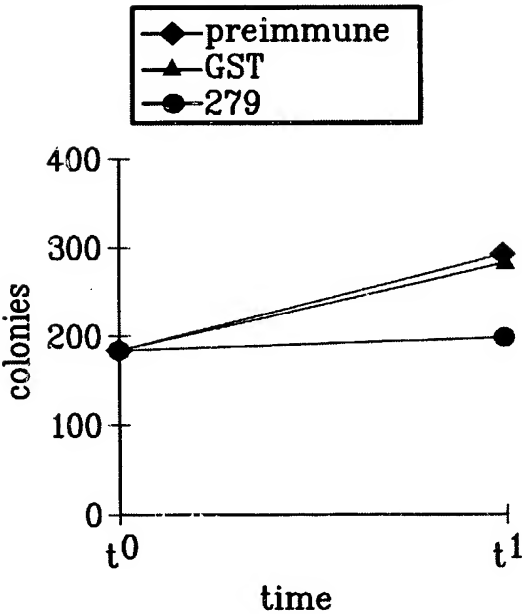


FIG. 2E

279 (10.5 kDa)
ELISA assay: positive

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FIG. 3A

576 (27.8 kDa)
PURIFICATION
M1 576

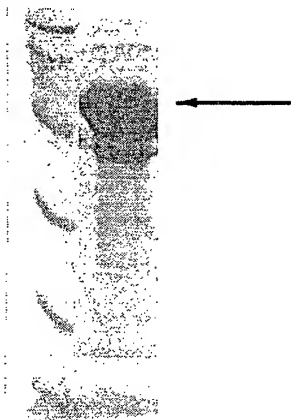


FIG. 3B

576 (27.8 kDa)
WESTERN BLOT
TP OMV

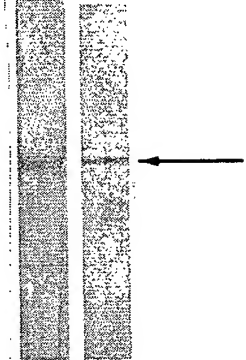


FIG. 3C

576 (27.8 kDa)
FACS

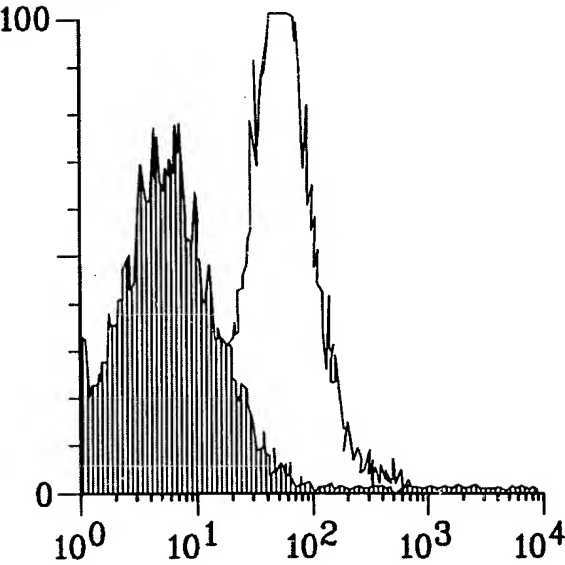


FIG. 3D

576 (27.8 kDa)
BACTERICIDAL ASSAY

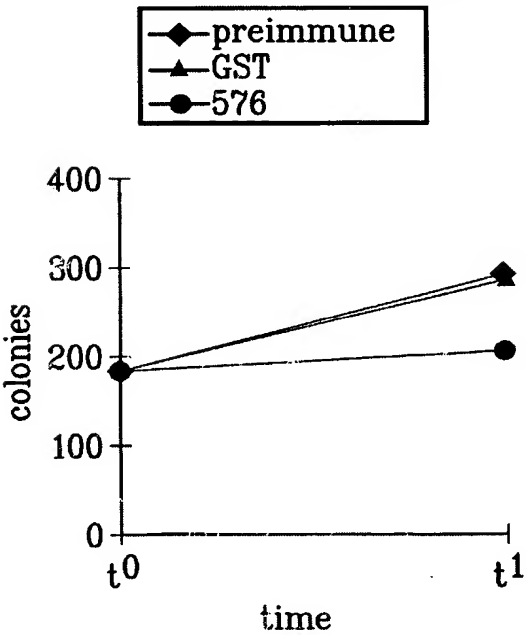
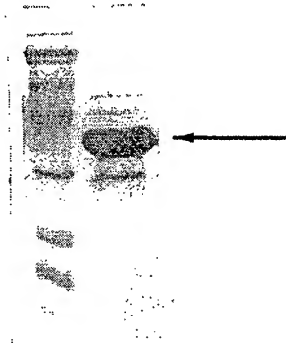
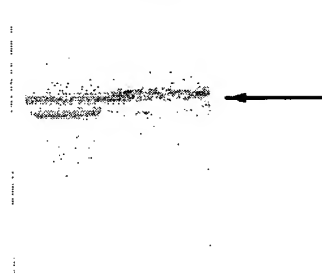
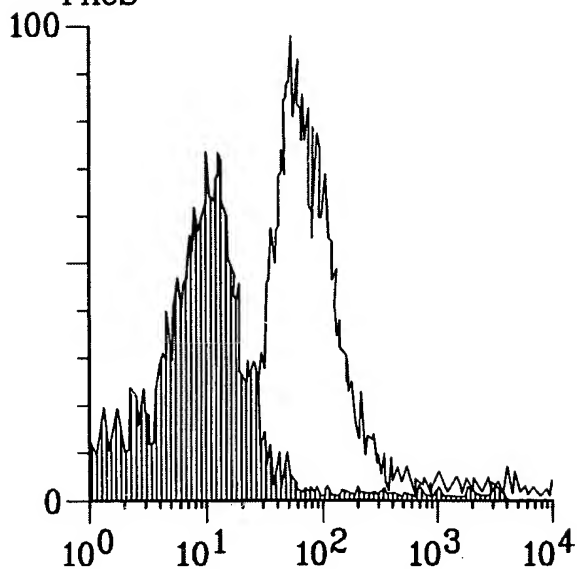
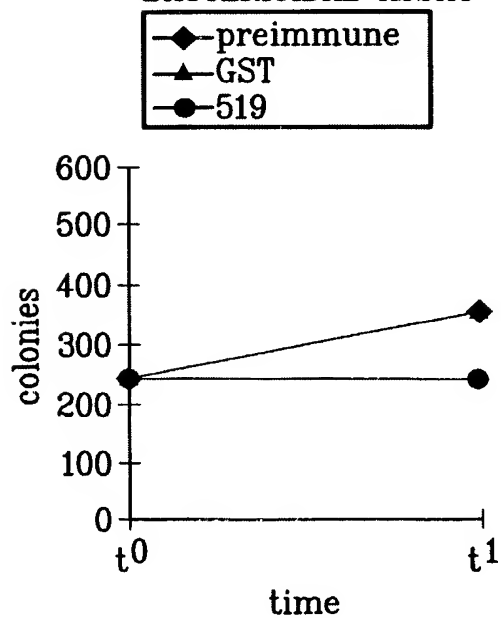


FIG. 3E

576 (27.8 kDa)
ELISA assay: positive

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*FIG. 4A*519 (33 kDa)
PURIFICATION
M1 519*FIG. 4B*519 (33 kDa)
WESTERN BLOT
TP OMV*FIG. 4C*519 (33 kDa)
FACS*FIG. 4D*519 (33 kDa)
BACTERICIDAL ASSAY*FIG. 4E*519 (33 kDa)
ELISA assay: positive

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FIG. 5A

121 (40 kDa)
PURIFICATION
M1 121

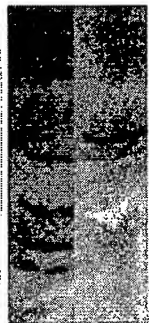


FIG. 5B

121 (40 kDa)
WESTERN BLOT
TP OMV



FIG. 5C

121 (40 kDa)
FACS

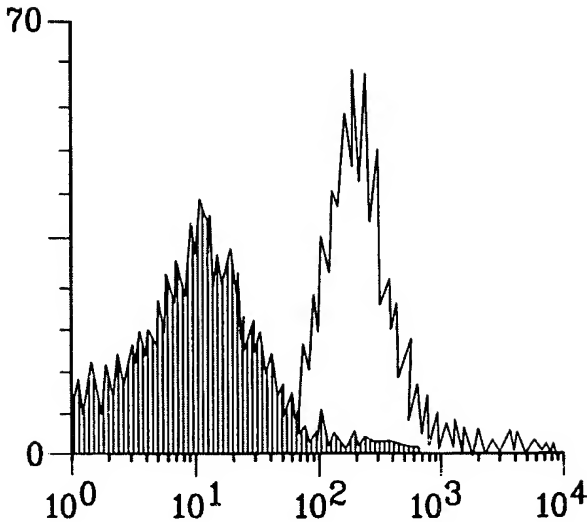


FIG. 5D

121 (40 kDa)
BACTERICIDAL ASSAY

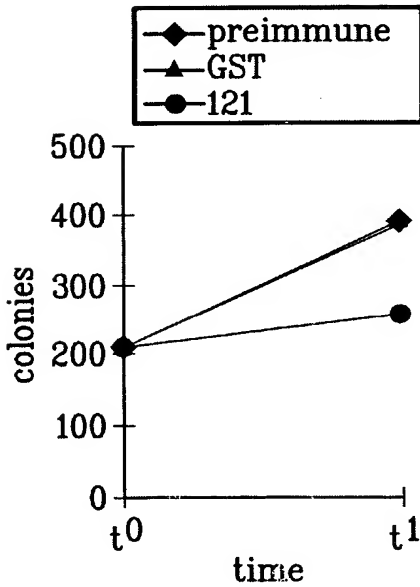


FIG. 5E

121 (40 kDa)

ELISA assay: positive

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FIG. 6A

128 (101 kDa)
PURIFICATION
M1 128

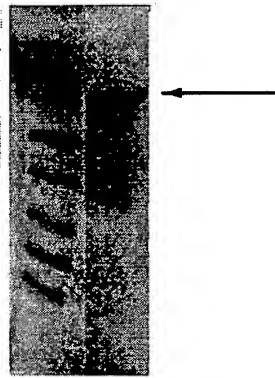


FIG. 6B

128 (101 kDa)
WESTERN BLOT
TP OMV

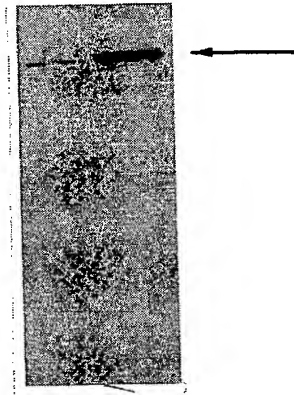


FIG. 6C

128 (101 kDa)
FACS

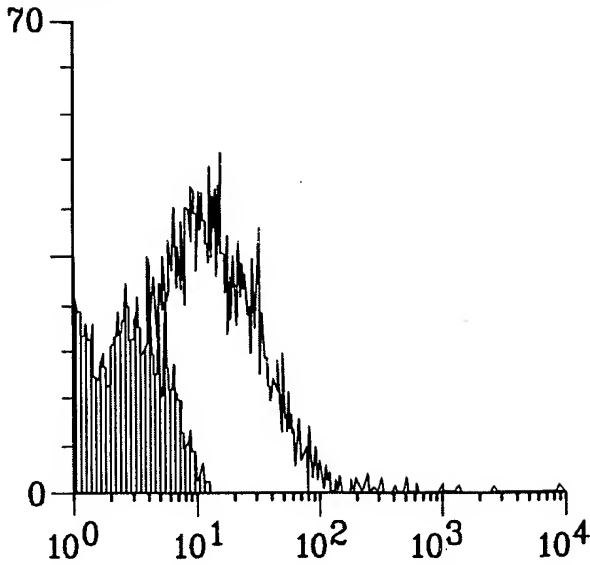


FIG. 6D

128 (101 kDa)
BACTERICIDAL ASSAY

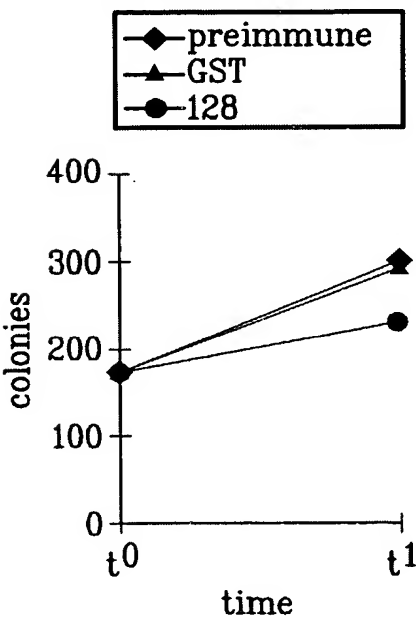


FIG. 6E

128 (101 kDa)
ELISA assay: positive

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FIG. 7A

206 (17 kDa)
PURIFICATION
M1 206

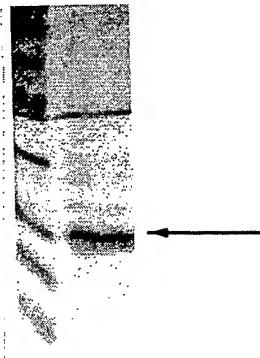


FIG. 7B

206 (17 kDa)
WESTERN BLOT
TP OMV

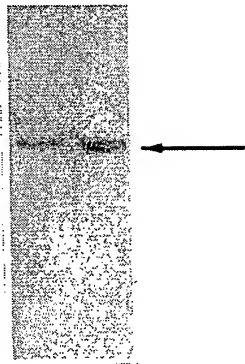


FIG. 7C

206 (17 kDa)
FACS

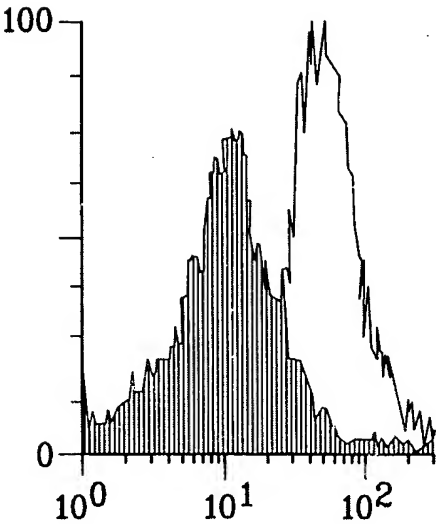


FIG. 7D

206 (17 kDa)
BACTERICIDAL ASSAY

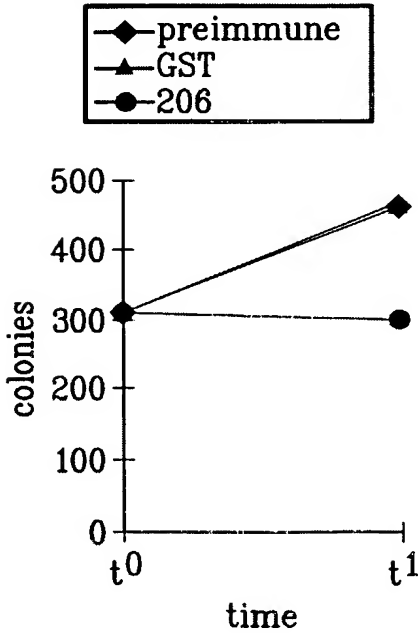


FIG. 7E

206 (17 kDa)
ELISA assay: positive

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FIG. 8A

287 (78 kDa)

PURIFICATION

M1 287

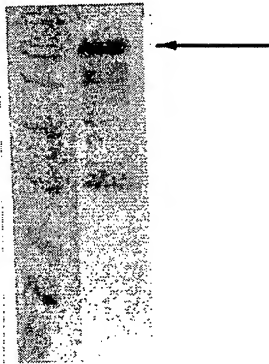


FIG. 8B

287 (78 kDa)

FACS

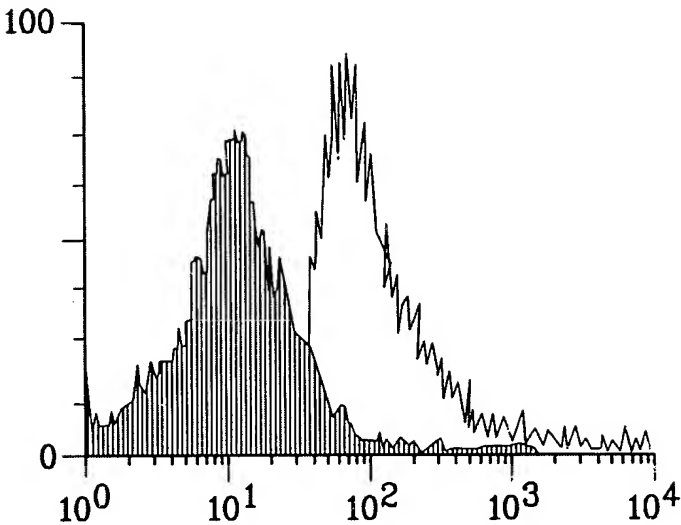


FIG. 8C

287 (78 kDa)

BACTERICIDAL ASSAY

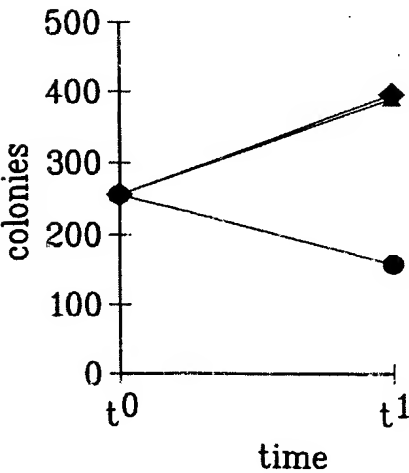
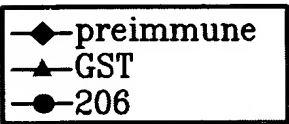


FIG. 8D

287 (78 kDa)

ELISA assay: positive

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FIG. 9A

406 (33 kDa)
PURIFICATION
M1 406

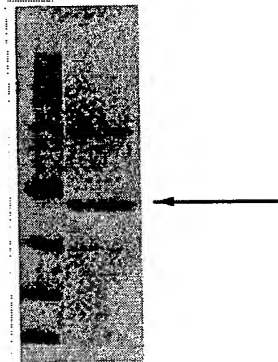


FIG. 9B

406 (33 kDa)
WESTERN BLOT
TP OMV

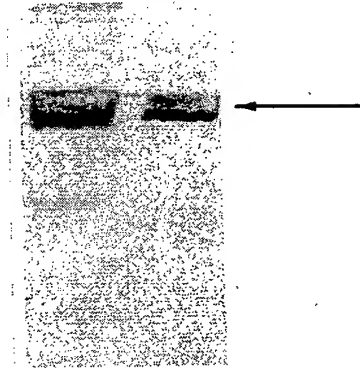


FIG. 9C

406 (33 kDa)
FACS

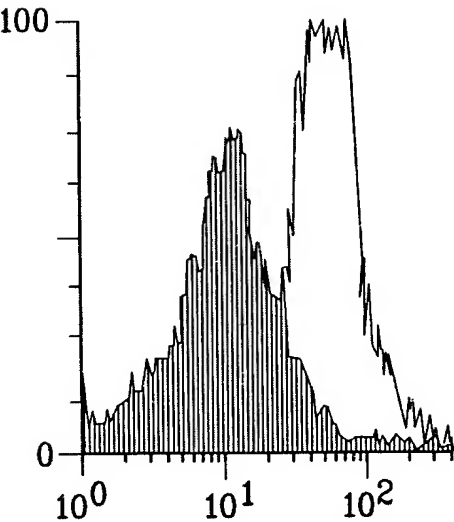


FIG. 9D

406 (33 kDa)
BACTERICIDAL ASSAY

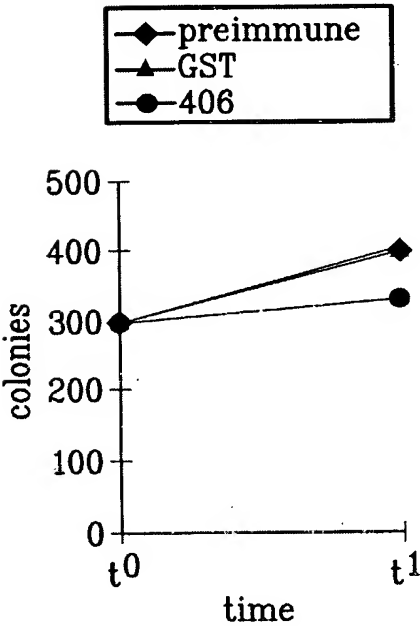


FIG. 9E

406 (33 kDa)
ELISA assay: positive

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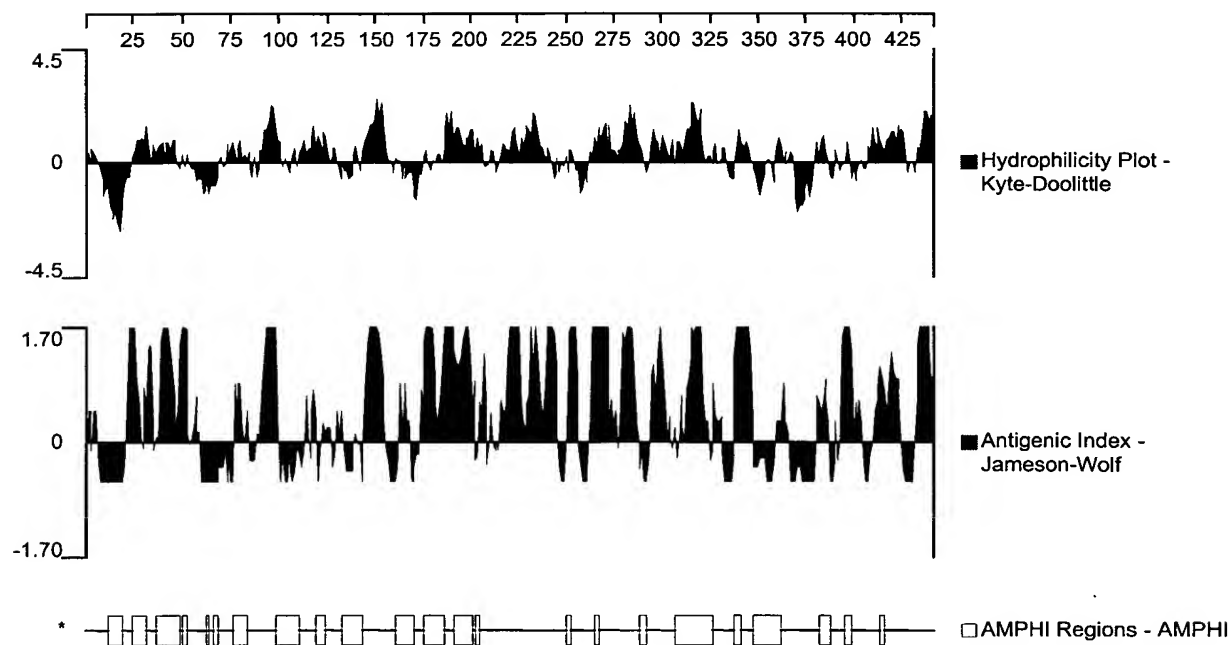
919Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 10

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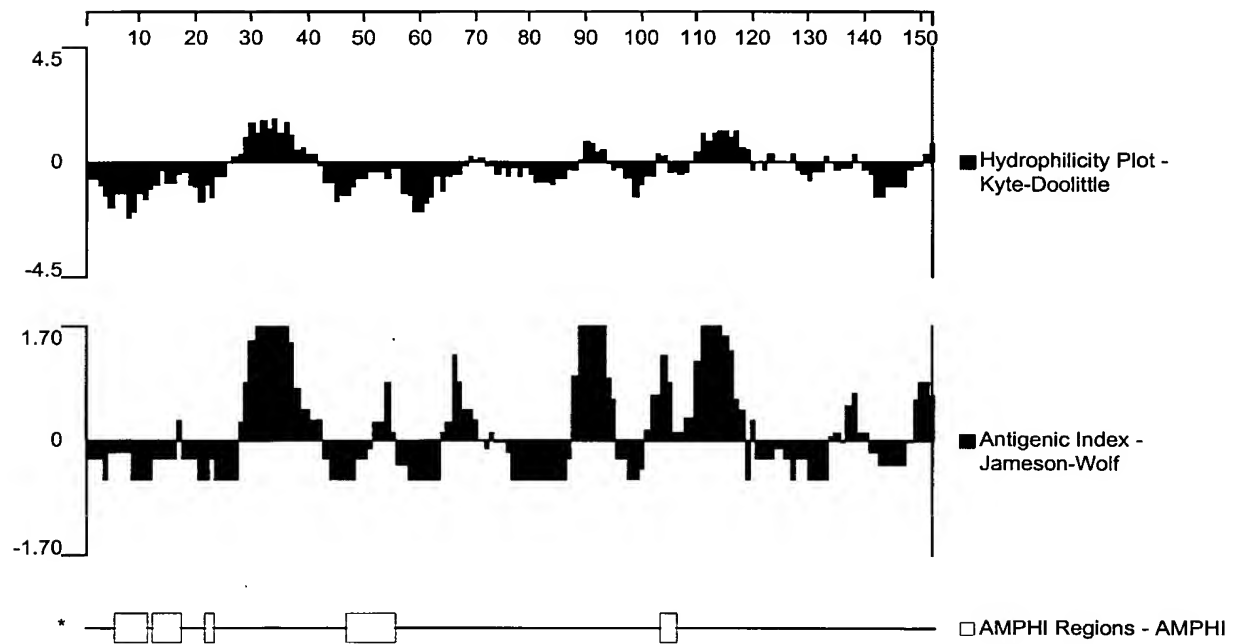
279**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

Fig. 11

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576-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

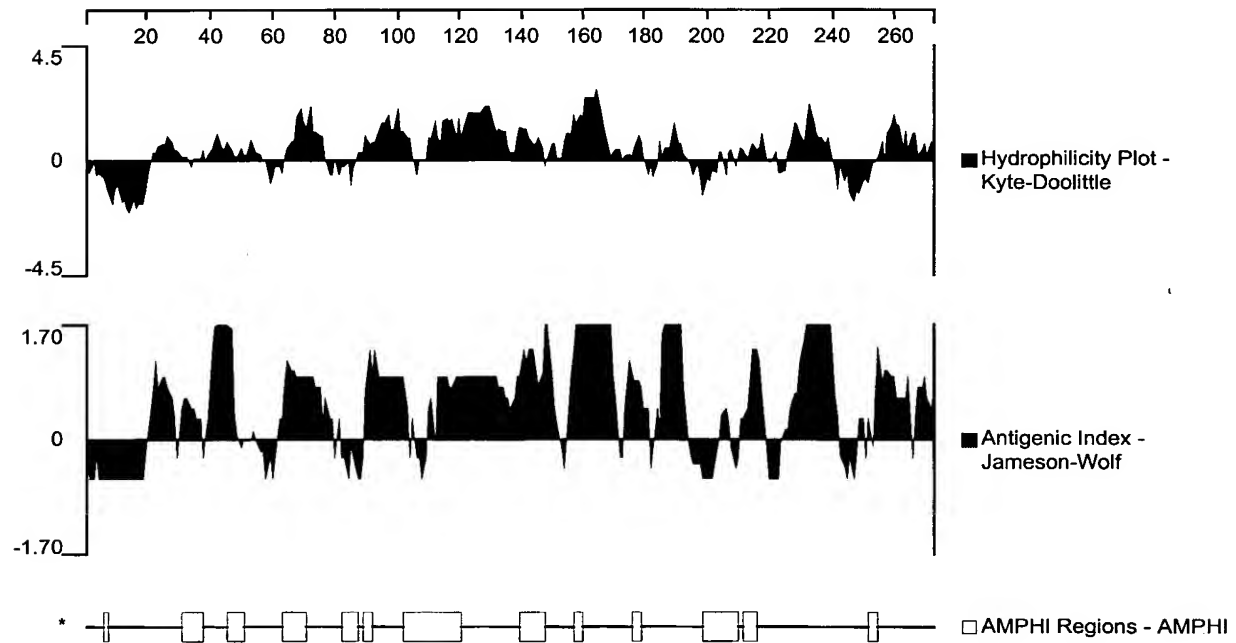


Fig. 12

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519-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

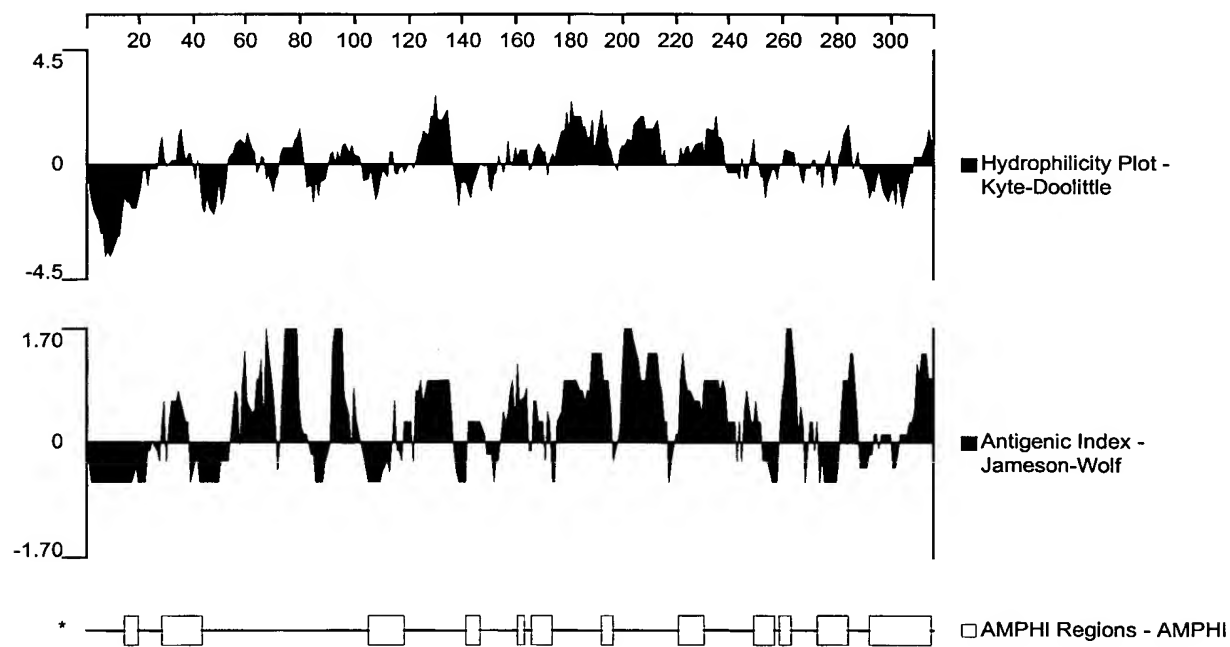


Fig. 13

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121-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

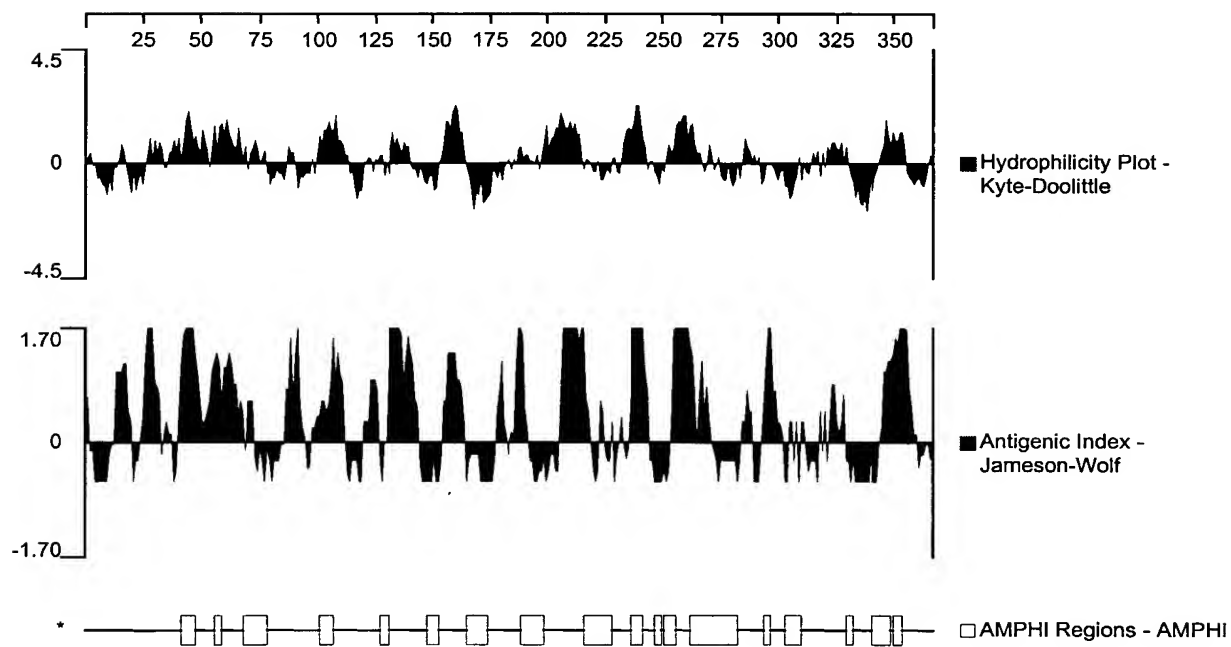


Fig. 14

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128-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

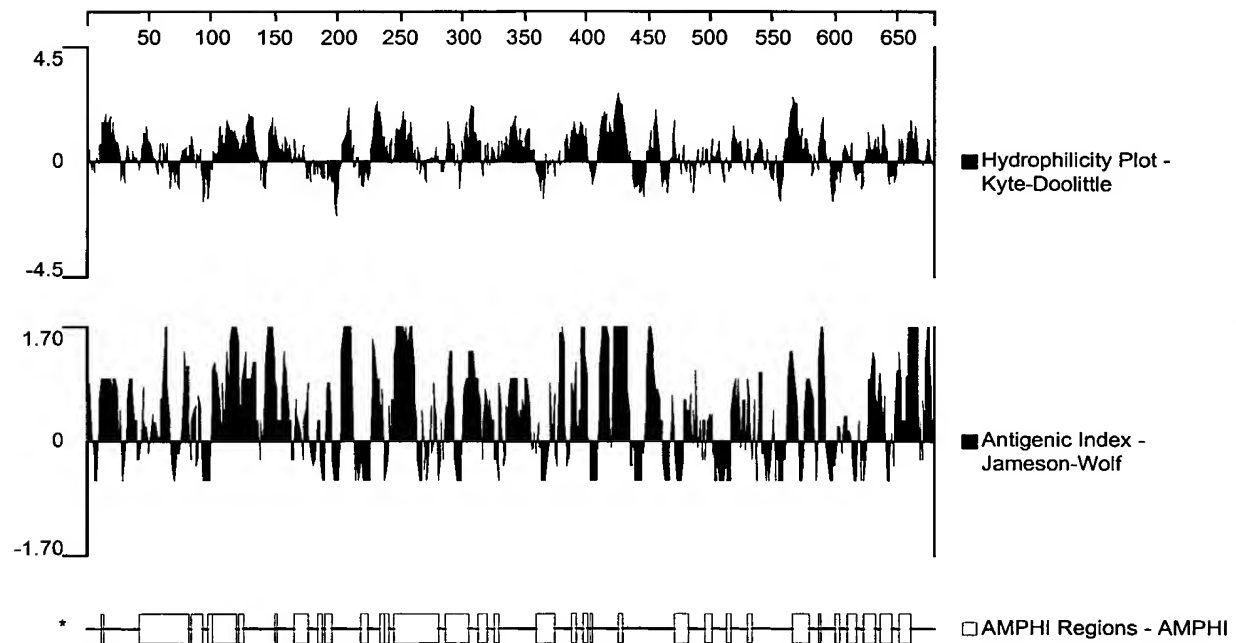


Fig. 15

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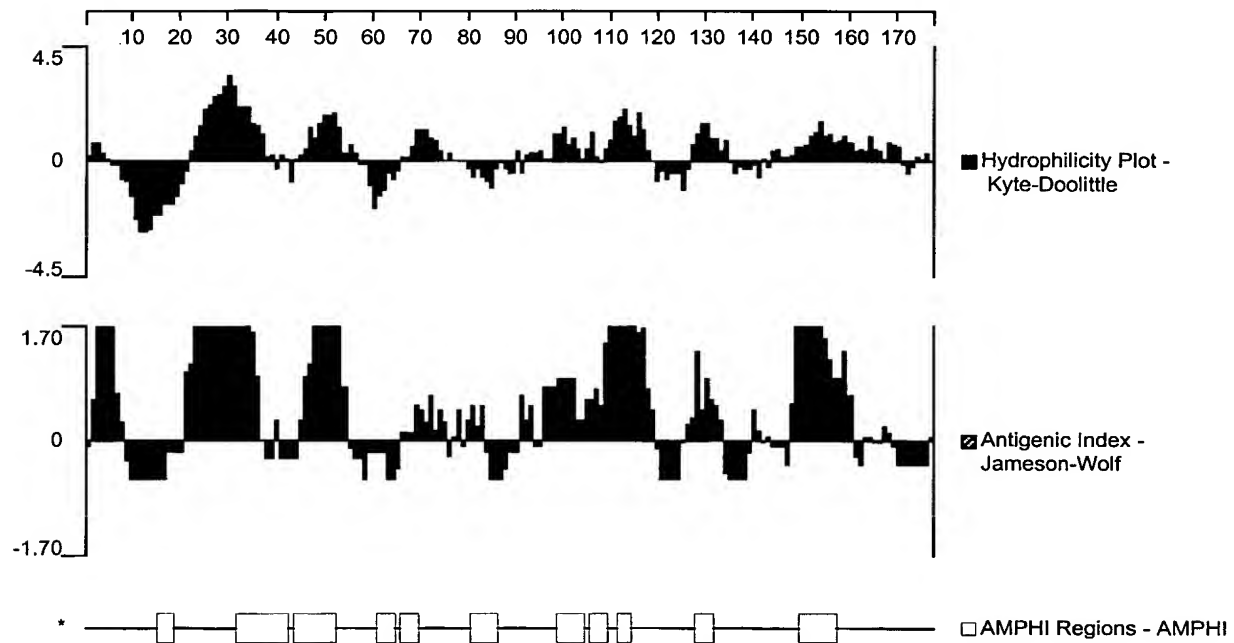
206Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 16

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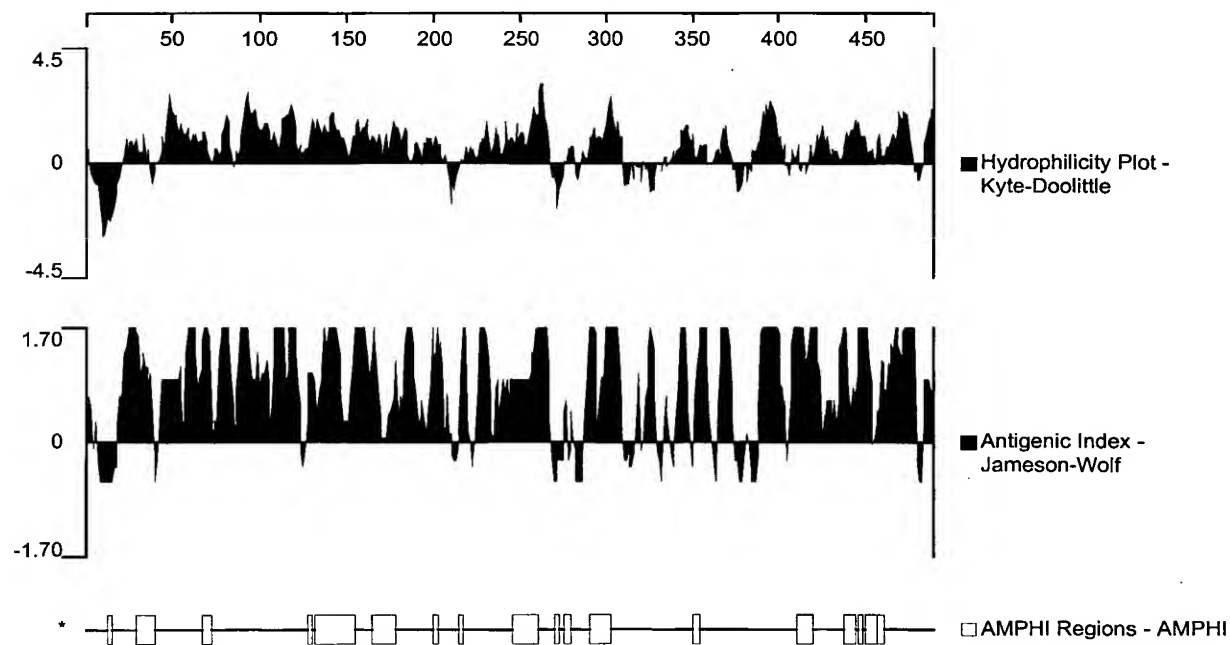
287Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 17

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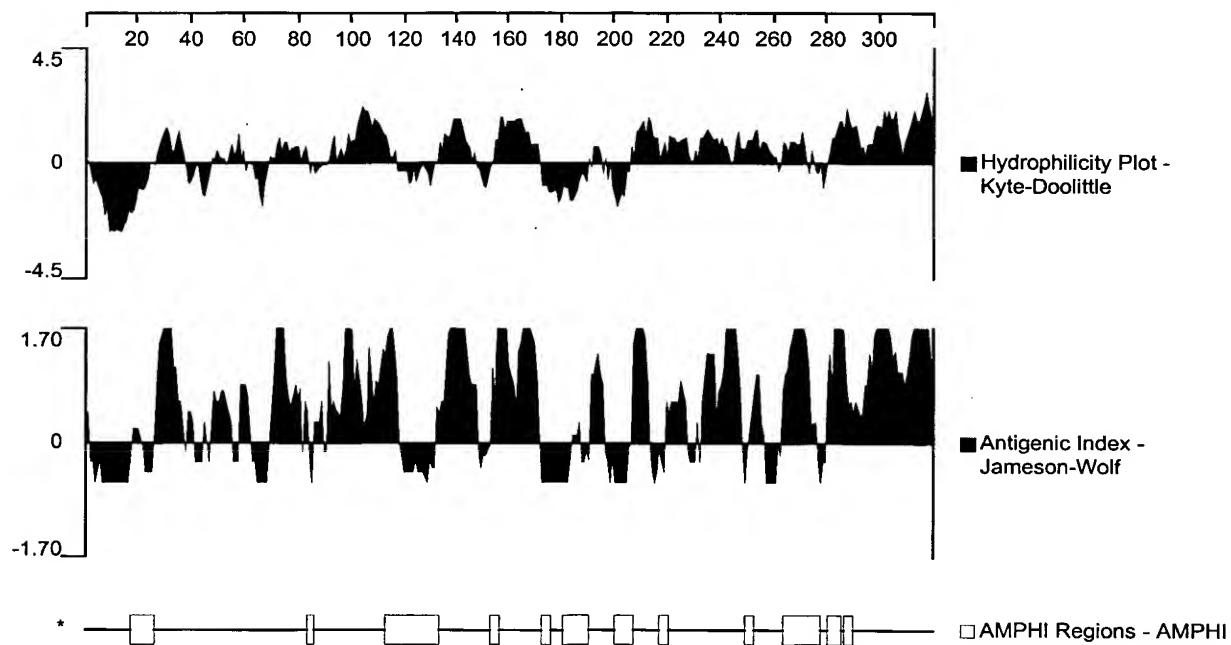
406Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 18

Appendix A

-1-

APPENDIX A

The following DNA sequence was identified in *N. meningitidis* B <SEQ ID NO. 1>:

TAAACCTTATCCACATCCAAACGCATAACCGTAACCCATTACCGTTATGGAAATGTCGC
CCGACAACCCAGCCGAATGATTCATAAAATATTGACATCAGGCGTATAAAGATAC
AAGAACCTTATCCCGAGCGAAGCGCTGCGCCTATGCAGTGGGCGACCGCCTCCTGCCA
ATGCCTTTTCCGCGATATTTCAGGTAAACAAAGACATCCCCAACCAATATTCATACCGT
GGAAACCTTTCCATATCATGCCGCTTGACCGCAGCCGAACCAACAGGATTCGGGAATCA
TCCACAGCCGCAAAATGCCAGCGGAGTTTCGTATCCTTCAAACACCTGCCGTAATAGGCA
TGAATCTTATCCACAGAAGACCACGGTTCAAATCCGTGCCACTCCTCAAACAACGCCTGA
ACCAACCTGCCGATATGCCCGGCTTTCAGCGGTGTAATGAAACAGTATTGTCCACAAAG
AGGGAATTCATCGGTCAATTCGCCGACGCCTTCGTTCCCGCTGCCCGTAACCGCATTC
CAAGCATGGTCCAAACGCACTCCGATTTGCTCAAATCTTCAGCCTGCCGGGCTTTTTCG
GCCATTGCTGCAGGAATTTCCGCTTCCAAACGGGCGATGTCTGCCTGAGCCGTCTGCAAA
CGCCGGCGCGCATCTTCCAAATCCGACTGCATCCCGATGATTTTCCGTCCAGATTGTTT
TGCTTTTGAATAAGCGCGGTAACCGGATGGATGCTGAGCAGATTGCTTCAGCATCC
CCTGCCCATACGCTTGTAGAAAAACAACCATCAGAAAAATAAATATTTTTTTCATTTTT
AATCTCCATTTAAATGCTGTCTGAAGCCGTATTCCGACATCAGACGGCATCGCCACGCGC
TGTGGATAACTTAAGCGCGGATGCGTTTCAACACTTCTTCTTTCGCGATTAAATGCCAACA
CAGCATCGACGCTGGGGGTTTTTCGCCGTACCGCAGACGGCAAGGCGCAGGGGCATGCCGA
GTTTGCCCATTTTAATGCTTCTTCGTGCGAGAAGGGTTTGAAGAGGTTCGTGGATGGCTT
CGGCATTCCAGTCTTCAGCCCTTCGAGGCGTTCGGCAAAGCGCAGCATACGGGCGGCGG
CTTCATCGTCCCATGTTTCTGCACGCTCTGCTTCGGCAGGCGTTTGTTCGCTAGAGT
AGAAGCACTCGTCGGCAAGCGTGTCAAGTCTTGGGGGCGGTCTTTCAGCAGTTCCAAACA
CATCTTCCAAAGCAGGTTTTTCGGTTTCAATGAATATCGCGCAACGCAAGGCGGGGTTGA
CGAGTTTCGGCGAGTTTTCGCGTTGGGTGTGATTTTGATGTGTTTCGCGGTGATCCAGTAGA
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CGCCGCTGCGTTTGGAGATTTTTTTCGCTTGTTCGTTAAGAAATCATCGGCAGGTGGCGGT
ATTCGGGCAGGTTTCGCGTTCGATGGCTTTTAAGATGTTGATTGTTTCGGCGTGTGTTCA
CATGGTTCGTCGCGCGGATAACGTGGGTAAACGCCCATGTCGTAGTCGTCTACGACAACGC
AGAAGTTGTAGGTTCGGCGTACCGTTCGGCGCGGGCGATAATCAGGTATCGAGTGCTTCGT
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CCGGACGCCAGCGCGGTGCTAAGTCGCGGAGCCTTCTTTTTCGCGTTTCTCACGCATGG
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AACGCGCCAAGTTCGGTGTCTTCAATACGTAATAGGAACTCGCCCTTATGATGGCGGGCAA
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GGGCGAAACGGGTTTTGACGGTTCATGATGGCTCCGAAATCTTTGAAAGCGTTATTTTAC
TGGTTTTTACCGTCTTGGGCATCAAAAATGCCGTCTGAACCTGCGTTCGCGATAAAGTTT
CAGACGGCATTTTCTTGTGTTTCAATGCTTCGGCACGGGAACAGTGTATCACGCGCCGC
CGACCGAATTCCTTCGGGATTGCGTCCAAAAAAAGTTCAATGAAACAGCTAATTGAAAA
AATCCCGCCCCCATTTTCCAAACGGTAGAGGGATAACGCATATCCCTCTTGCAGCATAA
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GGACACCCAATCTCCGCCATTTCCGCCGCCCGGCCAAATGGTAGGGATCGCTGACAA
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CGTACCGCGCCCGACCTCGGCTTCGGTCATATAGCCTTTTTTGGTCCGGCTCCCGTAA
ACACGATTTTGCTTACCTGCGGCTCTGATAAAGTGCATGGCATGGTTGATGCGTTTCGC
GGAAACAGGAGAAGGGCGTTTGTCCACGCGCGGCGCCCAACACGAGCGGCGATCCG
CCCGACATACGGCGGCAAAACCTGCCACCCGTCGATAAACCGCCCAACGGATGAGG
CAAACACGAGCAAAAGCGGAAAAACACTCAAACAGAAACGCCCAACAGGTAAAGCGCA
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TCTGCCGACAGCGCCTTACCGTTTTCGCAAAACAATCGGACGCTCCAACAGGGCGGGATG
ATCGGCGATGGCACGACGAGCGCGTCATTGTCAAATTTGGGGTTGTCAAACCCAATTC
CTTATACAAATCATCTTTCACGCGCATCATCCGCGCGCGGATGCCAAGCCCAATTTGTT
GAAAAATCTCTTCAATTCGGACAAGTCGGGCGGCGTATCCAATATTGACCACTTCGGC
AGCAATGCCGCTTCTTCAATAGGGACAAGGCGGCGACGCGATTGCTGCAACGCGGATT
GTGGAATTTTTGATTTAGGCATGACATTTCTTCTTCGACAAATCCCTTATTATC
GGCTTACACAGGTTTTTACTCAATATCCCGCTTACAACCGTACCAACGGTTTACAATAC
CCGAATCGACATACAAAGGACAAAACGATGAAATACTTGAATCTTCCCGCAATCACCTT
GCCGCCACATTTGCCGCACATACCGCTTCGGCAGACGAACCTGGCCGGATGGAAAGACAAC
ACCCCGCAAAGCCTGCAATCGCTCAAAGCCCCGTACGCATCGTCAACCTTTGGGCGACT
TGGTCCGGCCCGTTCGCGCAAGAGATGCCGTGCCATGTCCAATGGTACAAGCGCAGAAA
AAAGGCAGCGTCGATATGGTCCGCATCGCGCTCGACACATCCGACAATATCGGCAACTTC

Appendix A

-2-

CTCAACAAACTCTGTTTCTACCCGATTGGCGTTACACCGGGGCGAACAGCCGAAAC
TTTATGAAAACCTACGGAACACTGTCCGCGTACTGCCCTTTACCGTCGTGGAAGCACCG
AAATGCGGATACAGGCAGACCATTTACCGGGAGGTAAACGAAAAAGCCTGACCGACGCC
GTCAAACTCGCCCATTTCAAATGCCGTTAAACGCCGGATGCCGTCTGAAGCCGCTTCAGA
TGGCATTCTTTCTTTCCACCCGCTGCCGGTGCAAACCTATCCACTATCTAAAACAGGC
GGAATCTTTATAATCGGCACTGTCTTACCTATTGTTTCAGACGGCATATCCCTGCCGACGC
AACCGCCGAAACGATATGCCGCCCTTCTTACAGGACCTCTATGATCCGTTTCGAACA
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CAAAGGCGAAATGATATTTATCGCGGGACACTCCGGTTCGGGCAATCCACCATCTCAA
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AGACCACAAAATCTCTACGACCGCAACGCTCCTGCAAAACGTCATCTGCCGCTTCGGAT
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GGGAACCTACCGTATCGTTGTCGCGACATGACGAAACCTGATGGCGGACTACGGACACCG
CATCCTGCGCCTCTCGAAAGGACGACTCGCATGAGCATCATCCACTACCTCTCGCTGCAC
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Appendix A

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Appendix A

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Appendix A

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Appendix A

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Appendix A

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Appendix A

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Appendix A

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GCCATGCCGATCGAAGACTGATCGAATCGCGGGTACTCTTACCGAAEAAACACCAAGATG
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GCCTTTT CAGAATGTGGCGGCGGAGGGGATAGATGTGAGCCGTGCCGAAGCGAGGATAACC
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Appendix A

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TGTTGAAAGACGGCGGCTACTGGGCGGCGATGAAGGGCGTGTATCCGCAGGAAGAAATCG
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CCAGCGTACCGTGGGCGATATAGCGGCGGTGCGGCAATCTTCGGAATCAGACCAAGC
CTGCGGCTTTTCCGGCTTCCAAGAGCAGGCTGCCGGAAGTGCCATCAATTTCAATTTTCG
GGTCGAAATCCGCCCAATTCGGGCAACGGTATCCGCGCAGCGCACGGTTTTTTCGCGCTT
GATTGTACATTGCGCGGTGCGGTTTGACATAAGCCATTTCCAACCCCTGATCAGCGCACA
AGGCTGCAATGCGCCCACTGGTAATTCAGACACGCGCGCAATCGGCTTCGGACAGAT
TCATTTGCGTACGGCGAAGTTTTTCCGATCGGGATAGCGGGGTGTGCTCCGATGCGCA
CGCGTTTTTGTGGGCATACGCCAATGCCGCCGAATATCGGCAATGCTGCGGCGGTGTT
GGGCGCAGGCGATGTTGCCGAAGTAATCAGCTGCAACAAGGCTTCGTGCTGCGCGAGC
CTTCGGCGAGATCGGCTTTAAATCAACCTGCTTCATGGGTGATTCTCCGTATTGTGTTT
AGATAGGCTTGTTTTTGCGCGCAGGGCGGTGGCTTCTTTCAAGCCGATTATTTTGAATT
TGACTTTGCTGCGGAAGCGCACCTGTGCCAGCTGCCAAATCGCGCGGCGCAACGGTAG
CGATTTTCGGATAACCGCGCGGTGGTTTGGCATCGGCCAGCAGGATAATCGGTTTGGCGC
CGGGCGCACCTGCACGGTTCTGCTGACAGCGTGGGACAGCATTTCGAAGGTTGCG

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ACAGGGTCAGCGGCTGTCCGTGCAAGCGGTAGCCCATGCGGTTGCTATCGCTTTGCAGCG
TCCACGTTTCCCGTTCCAGATTTCAGACGCCCTTTTTCACAGAAAGCGGCATATTCCGACG
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CGCCGAAACCGGTTTCAGGTGCTTCTCGAACCCATCACTTCGGGCACATCAAAATC
CGCCCGCCACGCACACATAGCCGTACATGCCCTGCACGGCAGCACCAGTTTCAAGGTCT
GCCCTTTGCGGGCGGTATAACGCCAATACGAATAGACCGGTTGCGCGTCCAATTCGCCCT
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CCAAAGCAACCGTGTCCATCGCACCGGCATGACCGATGCGGTAACGCCGGTGTCCGTAGC
GTCCGGTATCCTGAATATGCGCCGGTGCCTGCCTGCGGAAACGTGAATCATGGCTCAAT
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